Natural Products

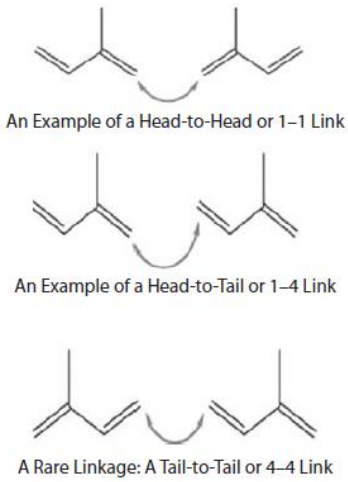
Natural products are **small molecules produced naturally by any organism including primary and secondary metabolites**. They include very small molecules, such as urea, and complex structures.

Terpenes

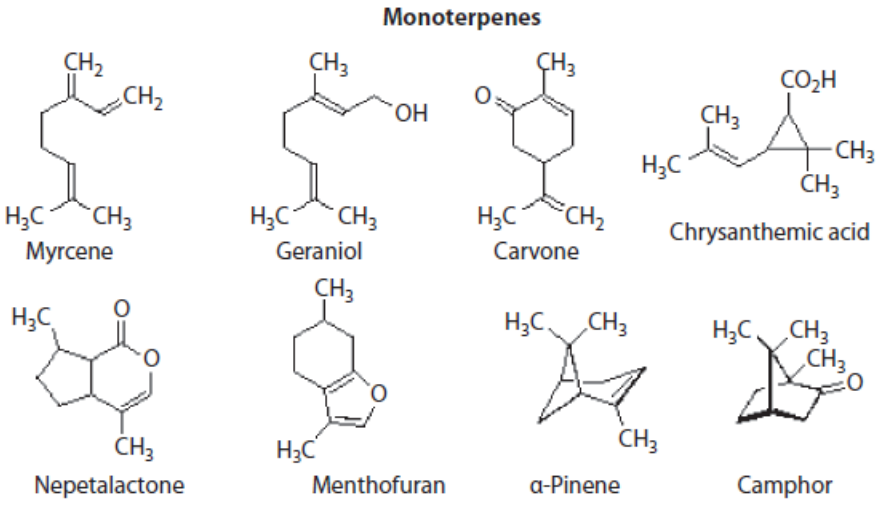
Terpenes may be defined as a group of molecules whose structure is based on a various but definite number of isoprene units (methylbuta-1,3-diene, named hemiterpene, with 5 carbon atoms). When terpenes are modified chemically, such as by oxidation or rearrangement of the carbon skeleton, the resulting compounds are generally referred to as *terpenoids*. Based on the number of building blocks, terpenoids are classified as monoterpenes, sequiterpenes, diterpenes, sesterpenes, triterpenes, tetraterpenes and poly terpenes.

Monoterpenoids represent a large class of terpene compounds. Biochemical modifications such as oxidation or a rearrangement reaction produce a variety of open chain and cyclized monoterpenoids; and several structures.

Monoterpenes consist of two isoprene units and have the general molecular formula $C_{10}H_{16}$. They are low molecular weight compounds, are very volatile, and many can be recognized by their distinctive odors.
Isoprenes are made from C-5 units possessing 5n carbon atoms; n is an integer. Two or more isoprene molecules are linked to one another to create terpenoids. Linking between two isoprene molecules can occur in three ways, either with the
“head” or “tail” of the molecule.



Different ways of connecting isoprene units.



Examples of naturally occurring oxygenated monoterpene structures.

The various general chemical properties of terpenoids are as follows:

1. They are unsaturated compounds (open chain or cycles) with one or more carbon atom rings having one or more double bonds. Consequently, terponids undergo addition reaction with hydrogen, halogens, halogen acids etc. some of them forms hydrates. They also form characteristic addition products with NO_2, NOCl and NOBr. These addition proudest are found to be useful in the identification of terpenoid. A number of addition products have antiseptic properties.

2. They undergo polymerization, also dehydrogenation in the ring.

3. As they have olifinic bonds, they are very easily oxidized nearly by the entire oxidizing agent.

4. A number of terpenoid are labile and hence readily isomerised in the presence of and into more stable forms.

5. On thermal decomposition, most of the terpenoids yield isoprene as one of the products.

Isoprene Rule:

Wallach, in 1887 enunciated the famous isoprene rule, which stated as follows: "The skeleton structures of all naturally occurring terpenoids are built up of isoprene units."

From the above rule it follows that the divisibility into isoprene units is regarded as a necessary condition to be satisfied by every naturally occurring terpenoid. The isoprene rule has been deduced from the following facts.

The empirical formula of almost all the naturally occurring terpenoid is C_5H_8

$(C_5H_8)\ n = \frac{distructive}{distilation}$ nC_5H_8 *isoprene*

The thermal decomposition of almost all terpenoids gives isoprene as one of the products. For example rubber on destructive distillation field isoprene as one of the decomposition products.

$(C_5H_8)\ n = \frac{distructive}{distilation}$ nC_5H_8 *isoprene*

Isoprene rule has been confirmed by the fact that under special experimental condtions, isoprene undergoes polymerisation to yield various terpenoids.

***EXTRACTION AND GENERAL METHODS OF STRUCTURE* DETERMINATION OF TERPENOIDS**

Due to their wide occurrence in nature, all the terpenoids could not be isolated and separated by a general method. However, mono-and sesqui-terpenoids have a common source, i.e., essential oils and, therefore, their isolation has been generalised. This is carried out in two steps as follows:

1. Isolation of essential oils.

2. Separation of terpenoids from essential oils.

Let us discuss these steps one by one.

a) **Extraction by means of volatile solvents.** This method is widely used in perfume industry. This method is generally used for such plants which yield an oil or give low quantities of oil on steam distillation due to decomposition of essential oils. In such cases, the plant material is directly treated with light petrol at 50^0C. Under these conditions the oil is taken up by the solvent along with the soluble colouring materials. The essential oils from this extract are separated by removing the solvent by distillation under reduced pressure.

(b) Adsorption in purified fats. This method is also known as enfleurage method and is widely

employed in France. By this method, the yield of the essential oil is generally higher. This method is used to extract a large number of essential oils like rose and jasmine.

In this method, the fat is warmed to 50^0C in glass plates. Then, the surface of the fat is covered with flower petals and it is allowed to be kept as such for several days until it becomes saturated with essential oils. Then, the old petals are replaced by fresh petals and this process is repeated. After removing the petals, the fat is digested with ethyl alcohol when all the oil present in fat is dissolved in alcohol. Some quantity of fat is also dissolved in alcohol. This can be removed by cooling the alcohol extract to 20^0C, when the fat separates out. The alcoholic distillate is then finally fractionally distilled under reduced pressure to remove the solvent.

Recently, the fat has been replaced by coconut charcoal due to its greater stability and higher adsorptive capacity. After keeping the coconut charcoal in contact with petals for a number of days, the charcoal is submitted to steam to get essential oils. This method is superior to the enfleurage method.

2. Separation of Terpenoids from Essential Oils. The essential oils obtained from the step 1 generally contain a number of terpenoids and these are separated by various physical and chemical methods.

a) Physical methods. The various physical methods are as follows:

(i) Fractional distillation methods. The various terpenoids present in essential oils are separated by fraction distillation method. The terpeniod hydrocarbons distil over first followed by the oxygenated derivatives. Distillation of the residue under reduced pressure yields the sesquiterpenoids and these are separated by fractional distillation.

On an industrial scale, specially designed stills are employed and an efficient condensing system is necessary to minimize loss of more volatile hydrocarbons.

(ii) Chromatography More recently chromatography in its various forms has been widely used both for isolation and separation of terpenoids.

In adsorption chromatography, the essential oil is made to flow through a particular adsorbent when the different types of terpenoids are adsorbed at different places on the adsorbent to form different chromatograms. Then, the various chromatograms are eluted by different solvent systems

to get different eluates (each eluate is having terpenoids of a single group). Each eluate is then subjected separately to adsorption chromatography when different subjected separately to adsorption chromatography when different bands due to the various terpenoids present in eluate are obtained which are them eluted to yield different terepenoids.

In adsorption chromatographic method, alumina and silica gel are generally used as adsorbents for separating the terpenoids, particularly triterpenoids.

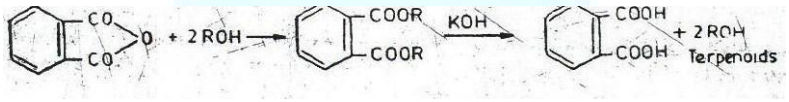
Gas chromatography has been particularly useful for isolating pure configurationally forms of a given terpenoid from mixtures produced by synthesis.

b) Chemical methods. These methods are not used these days to separate various terpenoids from essential oils. However, the various chemical methods are as follows:

i) When essential oils containing terpeniod hydrocarbons are treated with nitrosyl chloride in chloroform, crystal line adducts of hydrocarbons having sharp melting points are obtained. These are separated and decomposed into their corresponding hydrocarbons.

ii) When essential containing alcohols are treated with phthalic anhydride to form diesters, the primary alcohols react with phthalic anhydride readily, secondary alcohols less readily and tertiary alcohol does not react at all.

After extracting with sodium bicarbonate, diesters are decomposed by alkali to the parent terpenoid alcohols.



iii) Terpenoid aldehydes and ketones are separated from essential oils by forming their adducts with the common carbonyl reagents like $NaHSO_3$, 2-dinitrophenylhydrazine, phenylhydrazine, semincarbazide, etc. After separation, these are decomposed to regenerate terpeniod aldehydes and ketones.

6. General methods for the determination of structure of terpenoids.

The fundamental researches done by Wallach, Baeyer, Perkin, Semmler, Simonson, Ruzicka, etc. are of great importance in elucidating the complicated structures of terpenoids.

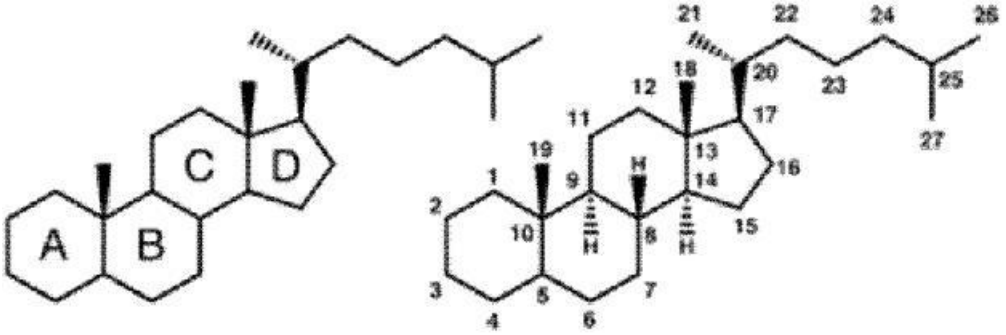
All the methods used for these have been grouped into four classes:

1. Analytical methods.
2. Synthetical methods.
3. Physical methods.
4. Knowledge of a molecular rearrangement.

STEROIDS

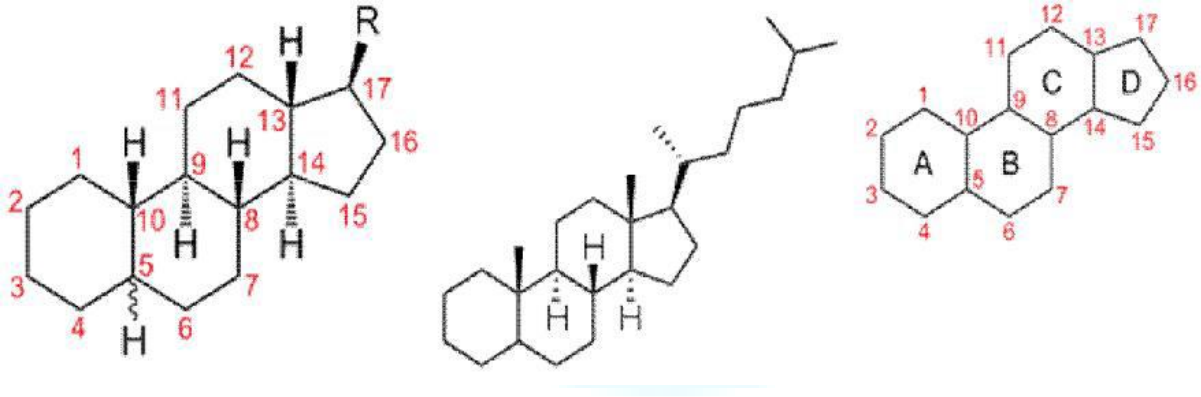
steroid is a type of organic compound that contains a specific arrangement of four cycloalkane rings that are joined to each other. Examples of steroids include the dietary fat cholesterol, the sex hormones estradiol and testosterone, and the anti-inflammatory drug dexamethasone.

Hundreds of distinct steroids are found in plants, animals, and fungi. All steroids are made in cells either from the sterols lanosterol (animals and fungi) or from cycloartenol (plants). Both lanosterol and cycloartenol are derived from the cyclization of the triterpene squalene.

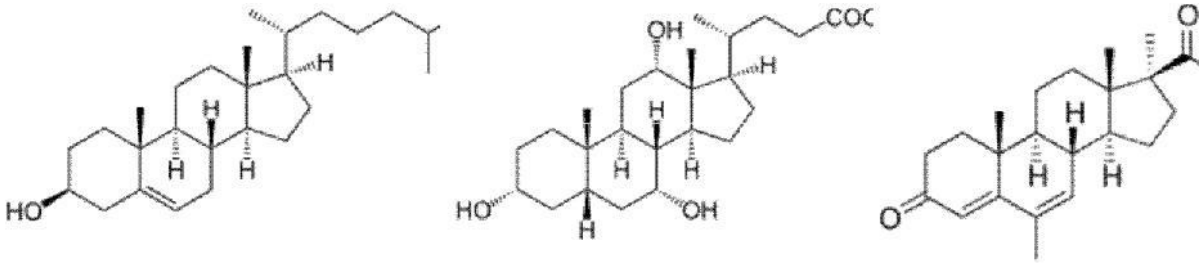


Structure of Steroid

Steroids are a class of organic compounds with a chemical structure that contains the core of gonane or a skeleton derived therefrom. Usually, methyl groups are present at the carbons C-10 and C-13. At carbon C-17 an alkyl side chain may also be present.

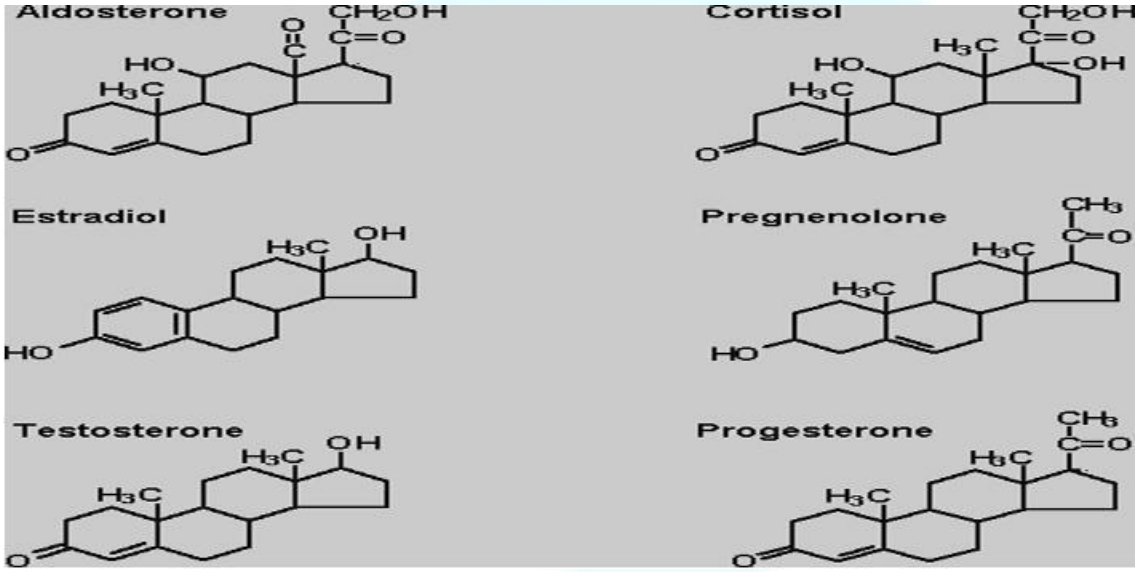


Gonane is the simplest possible steroid and is composed of seventeen carbon atoms, bonded together to form four fused rings. The three cyclohexane rings (designated as rings A, B, and C in the figure above right) form the skeleton of phenanthrene; ring D has a cyclopentane structure. Hence, together they are called cyclopentaphenanthrene. Commonly, steroids have a methyl group at the carbons C-10 and C-13 and an alkyl side chain at carbon C-17. Further, they vary by the configuration of the side chain, the number of additional methyl groups and the functional groups attached to the rings. For example the hydroxyl group at position C-3 in sterols.



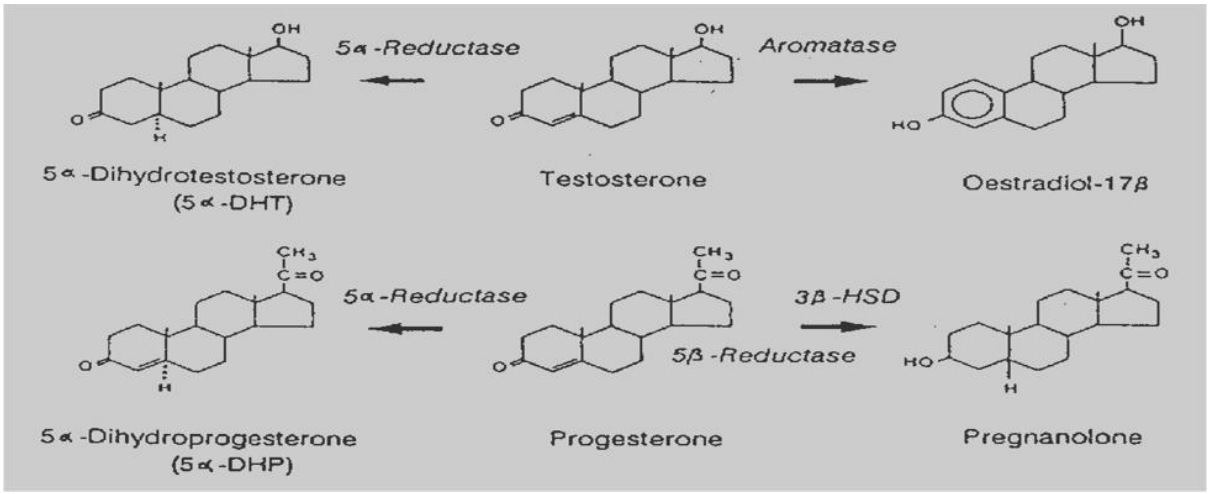
Metabolism of Steroid

Steroids include estrogen, cortisol, progesterone, and testosterone. Estrogen and progesterone are made primarily in the ovary and in the placenta during pregnancy, and testosterone in the testes. Testosterone is also converted into estrogen to regulate the supply of each, in the bodies of both females and males. Certain neurons and glia in the central nervous system (CNS) express the enzymes that are required for the local synthesis of pregnane neurosteroids, either *de novo* or from peripherallyderived sources. The rate-limiting step of steroid synthesis is the conversion of cholesterol to pregnenolone, which occurs inside the mitochondrion.



Steroid metabolism is the complete set of chemical reactions in organisms that produce, modify, and consume steroids. These metabolic pathways include:
steroid synthesis – the manufacture of steroids from simpler precursors
steroidogenesis – the interconversion of different types of steroids

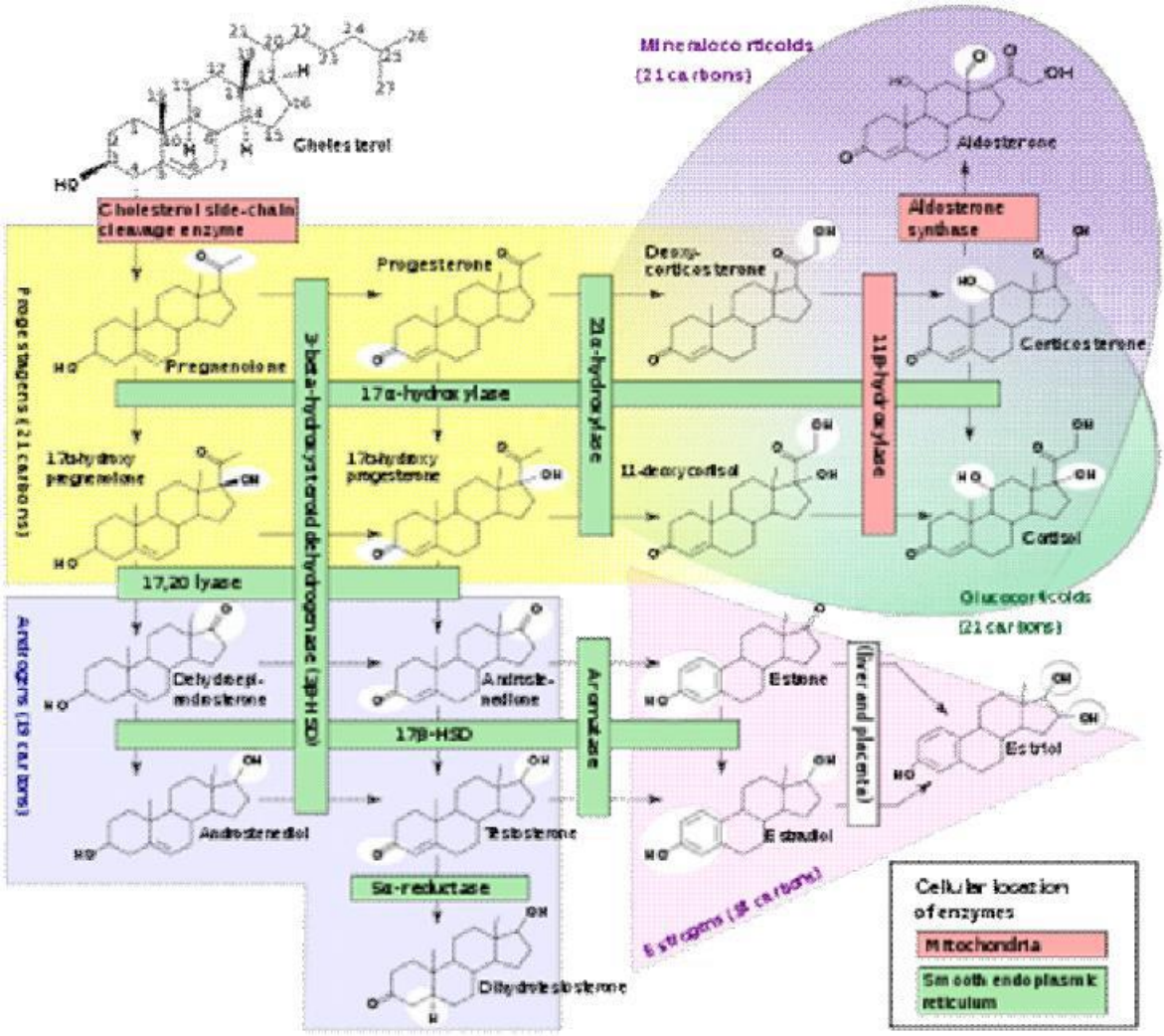
Steroid degradation.



Steroidogenesis

Steroidogenesis is the biological process by which steroids are generatedfrom cholesterol and transformed into other steroids. The pathways of steroidogenesis differ between different species, but the pathways of human steroidogenesis are shown in the figure.

Lanosterol can then be converted into other steroids such as cholesterol and ergosterol.



Human Steroidogenesis
Products of steroidogenesis include:
i. androgens: testosterone
ii. estrogens and progesterone
iii. corticoids: cortisol and aldosterone

Degradation and Elimination of Steroids
Steroids are oxidized mainly by cytochrome P450 oxidase enzymes, such as CYP3A4. These reactions introduce oxygen into the steroid ring and allow the structure to be broken up by other enzymes, to form bile acids as final products. These bile acids can then be eliminated through secretion from the liver in the bile. The expression of this oxidase gene can be up-regulated by the steroid sensor PXR when there is a high blood concentration of steroids.

ALKALOIDS:

Introduction: These are the basic nitrogenous compounds of vegetable usually having a marked physiological action and which may be regarded divided form parole, pyridine quinoline, isoquinoline or similar cyclic nitrogenous nuclei. Many of them possess curative properties and are of great value in medicine. **Occurrence:** Generally they are usually found in plants in the form of their salts- in which they are either combined with organic acids such as lactic, citric, malic, oxalic commonly found plants or with certain characteristic acid such as quinic acid and meconic acid.

In some case they exist glycoside. Generally they accumulate in the first and seeds and some times in the break of the trees.

Isolation of Alkaloids Plants: Alkaloids are extracted form plarite the plant material is finally powdered and treated with water acidified with HCL, when alkaloids form salts with HCL dissolve in water. The water extract contains the hydrochlorides of the Alkaloids together with Carbohydrates and other products form the plant tissue and free alkaloids are obtained from the acidified water extract which when treated with alkali precipitate out the alkaloids (being sparingly soluble in water) in the case of volatile alkaloids, the acidulated water extract is treated with alkali and stream - distilled.

Purification of the crude product obtained above is carried out by special methods or frequently by crystallization of the freed compounds or their salts.

General Properties:

(i) State: Most of the alkaloids are crystalline solids which cannot be distilled. Only a few of them are liquids and can volatilize without decomposition, e.g., coniine and nicotine.

(ii) Physiological action: Most of them are bitter in taste and often exert a marked physiological action.

(iii) Solubility: Almost all of them are either insoluble or sparingly soluble in water. Liquids alkaloids (coniine and nicotine are notable exceptions (being readily soluble in water and appreciably volatile in steam.) Alkaloids are generally less soluble in chloroform, either and benzene but are readily soluble in alcohol.

(iv) Optical activity: Most of them are optically active and usually laevo- rotatory.

(v)Basic nature: In a number of cases their solutions give a strong alkaline reaction. All of them form salts with acids, among these salts the chlorides, sulphates and oxalates crystallize well, there chlorides give double salts with chlorides of gold, platinum and mercury.

(vi) Precipitation : Alkaloids are precipitated form their aqueous or acid solution by a number of substances such as picric acid, tannic acid, perchloric acid, potassium mercuric iodide, potassium bismuth iodide, potassium bismuth iodide, phosphomolybedic acid and phosphotungstic acid. Precipitation with these reagents is often employed for the isolation and purification of alkaloids. This procedure cannot, however, be used for quantitative analysis since the resulting compounds are not sufficiently insoluble and because the reagents precipitate some other organic substances as well.

Determination of the Chemical constitution of Alkaloids: Different steps involved in the determination of constitution of an alkaloid are:

(a) Determination of Molecular Formula: The sample is purified and subjected to qualitative analysis. Carbon, hydrogen and nitrogen are invariably present while oxygen is rarely is rarely absent. This is followed by quantitative analysis, determination of molecular weight and then calculation of empirical and molecular formula.

(b) Ditection of Groups: Knowing the presence of nitrogen and/or oxygen in the alkaloid, the functional nature of these elements is determined.

FUNCTIONAL NATURE OF OXYGEN

(1) Hydroxyl Group: The alkaloid is treated with acetic anhydride, acetyl chloride or benzoyl chloride to detect the presence of hydroxyl group.

The hydroxyl group present my be phenolic or alcoholic. It is phenolic if the alkaloid -

(i) Gives a colour with ferric chloride:

(ii) Is soluble in sodium 'hydroxide and is reprecipitated by carbon dioxide.

If the hydroxyl group is not phenolic, it must be alcoholic this is confirmed by treatment with dehydrating agents (eg. H_2SO_4 or O_4O_{10}) or by oxidation.

(2) Carboxyl group: Presence of a carboxyl group is indicated by the solubility of the alkaloid in aqueous sodium carbonate or formation of esters.

(3) Ester group: Identification of the products of hydrolysis of alkaloid indicates the presence or absence of an ester group.

(4) Methoxy group: The presence of methoxy groups and their number is determined by Zeisel method which is described under Estimation of groups.

FUNCTIONAL NATURE OF NITROGEN

(5) Amino Group.

(i) The reactions of the alkaloid with acetic anhydride, benzoyl chloride, nitrous acid and methyl iodide show whether the amino group is primary, secondary or tertiary.

(ii) Formation of methylamine, dimethylamine, trimethylamine (volatile products) on distillation with aqueous potassium hydroxide indicates the nature and number of methyl groups attached to nitrogen atom.

(6) Amide group: Products of hydrolysis (acid and ammonia) of the alkaloid will show the presence of an amide group.

(7) Presence of Unsaturation: It's in an alkaloid sample is indicated by the treatment with bromine water or dilute alkaline permanganate.

(c) Estimation of Groups: The estimation of various groups, detected as above, is carried out as follows.

(1) Hydroxyl groups: The number of hydroxyl groups is determined by acetylating the alkaloid followed by hydrolysis of the acetyl derivative with a known volume of N-NaOH. The excess of the alkali left unused is estimated by back titration with a standard acid.

$$\underset{\text{Hydroxy Compound}}{ROH} \xrightarrow{CH_3COCl} \underset{\text{Acetyl derivative}}{RO.OCCH_3} \xrightarrow{NaOH} ROH + CH_3COONa$$

From the volume of N-NaOH used, the number of acetyl groups or hydroxyl groups can be calculated.

(2) Carboxyl groups. The number of carboxyl groups in a given sample may be determined volumetrically by titration against standard barium hydroxide solution using phenolphthalein as an indicator or gravimetrically by the silver self method.

(3) Methoxy groups: The presence of methoxy groups and their number many are determined by the Zeisel's method. The alkaloid is treated with concentrated hydroiodic acid at 399 K

(boiling point of HI). The methoxy groups present in the molecule are thereby changed into methyl iodide which is absorbed in alcoholic silver nitrate when silver iodide precipitated.

$$R(OCH_3)x + xHI \longrightarrow R(OH)x + xCH_3I$$

$$xCH_3I + xAgNO_3 \longrightarrow xAgI + xCH_3NO_3$$

The precipitate of AgI is boiled with HNO_3, filtered, washed, dried and weighed.

(d) Degradation: The complex molecule is broken into relatively simple fragments whose nature gives useful information about the type of nuclei present in the molecule. Various methods employed for degradation of an alkaloid are:

(i) Hydrolysis: Molecules containing an ester or amide group break on hydrolysis into simpler products. For example, piperine on hydrolysis splits up to give piperic acid and piperidine.

From this we infer that piperine is a piperidinamide of piperic acid.

$$C_{11}H_9O_2CO\text{-}NC_5H_{10} + H_20 \longrightarrow C_{11}H_9O_2COOH + C_5H_{10}NH$$

Piperine Piperic Acid Piperidine

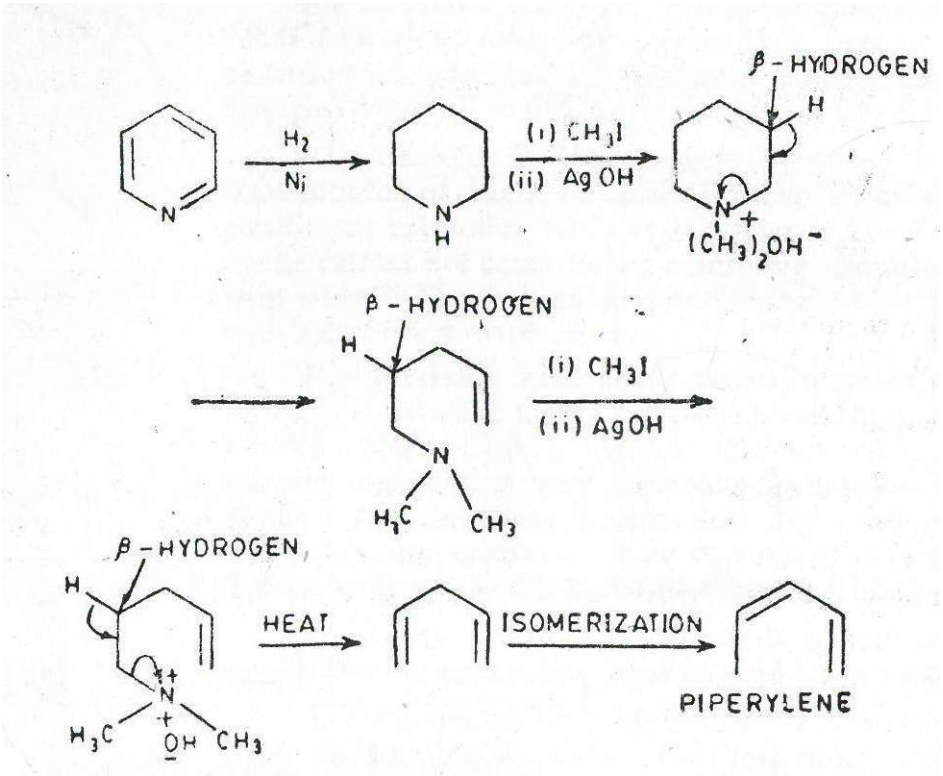
(ii) Oxidation: Alkaloids on oxidation give a variety of products depending the nature of oxidizing agents-mild (H_2O_2 or alkaline potassium ferricyanide), moderate (acid or alkaloid $KMnO_4$) or vigorous (K_2Cr_2O H_2SO_4; conc. HNO_3 or MnO_2 + H_2SO_4)

(iii) Distillation with Zinc dust: This brings about degradation or dehydrogenation of the alkaloid under study. When the alkaloid contains oxygen it is removed during distillation. For example, on distillation with zine dust morphine yields phenanthrene (parent compound) while coniine undergoes dehydrogenation to give conyrine.

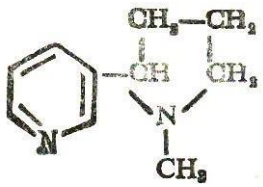
(iv) Hofmann Exhaustive Methytation: Heterocyclic rings containing nitrogenare opened with the elimination of nitrogen when subjected to exhaustive methylation. It thus helps us in knowing the nature of the carbon skeleton.

The heterocyclic, if unsaturated, is hydrogenated, and converted to the quaternary methylammonium hydroxide. This on heating loses a molecule of water by combination of -OH group with a hydrogen atom in B-position with respect to the nitrogen atom and the ring is opened at the nitrogen atom.

On repeating the process with the product, nitrogen atom is completely removed and an unsaturated hydrocarbon is left behind which generally isomerises to a conjugated diene. For example, starting with pyridine we have:



(d) Synthesis. The alkaloid under investigation is assigned a tentative structure on the basis of the foregoing analytical data. This is finally proved only if it could be synthesised by a suitable unambiguous method.



NICOTINE ($C_{10}H_{14}N_2$):

It is the chief alkaloid of the tobacco plant (Nicotiana tobacco) where in it is present as a salt of malic or citric acid. In leaves of tobacco its concentration is the highest. It varies from 0.6 to 8% depending upon the kind of tobacco.

The alkaloids are conveniently prepared from tobacco leaves. Raw tobacco of high nicotine is crushed and its soluble constituent extracted with cold water. The hydrocarbons present in the extract are removed by acidifying the solution and extracting with ether. The residual solution is made alkaline and nicotine sec free is extracted with other.

Properties: Freshly prepared nicotine is a colourless oily liquid. (b.p. 519.K under 730 mm pressure) readily soluble in water. Unlike tobacco, pure nicotine has an unpleasant smell. It has a burning taste and is very poisonous (lethal dose being 30 to 50 mg). In air it rapidly turns brown and resinifies and cab be distilled without decomposition only in vacuum or in a current of hydrogen. The natural alkaloid is laevo-rotatory and has [x] of - 169^o

In a mixture with soap solution it is one of the most effective exterminating agents for green fly and other insect pests.

Constitution:

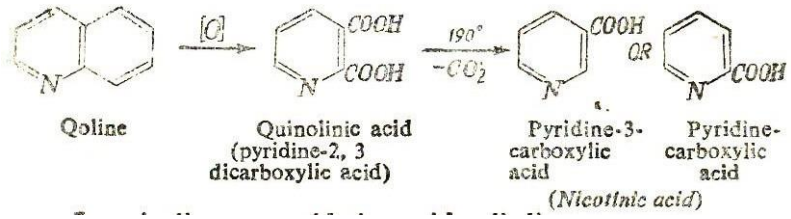
(1) Molecular formula of nicotine as deduced from its analytical data and molecular mass determination is $C_{13}H_{14}N_2$.

(2) Nicotine reacts with methyl iodide to form dimethiodide and two monomethiodidis but it does not form an acetyl or benzyl derivative. This show hat the two nitrogen atoms in nicotine are tertiary.

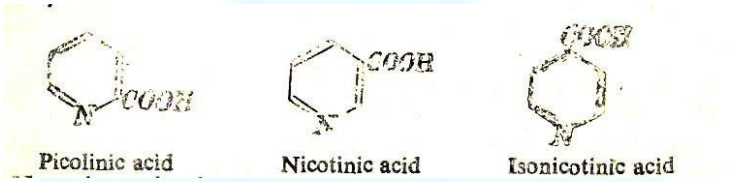
(3) Nicotine on oxidation with chromic acid or permanganate gives nicotinic acid ($C_6H_4N_2COOH$). Three pyridine carboxylic acids are known with COOH group in 2-,3- or 4-position. These are named picolinic acid, nicotinic acid. Their orlentation was proved as follows:

Quinoline on oxidation with alkaline permanganate gives quinolinic acid which must be pyridine-2, 3-dicarboxylic acid. Quinolinic acid on being heated to 360K loses one carboxyl

group and gives nicotinic acid. Hence nicotinic acid must be either pyridine-2-carboxylic acid or pyridins-3-carboxylic acid.



By elimination, therefore, picolinic acid is pyridine 2-carboxylic acid.



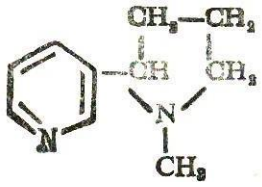
Now since nicotine on oxidation followed by heating at 460K yields nicotinic acid (pyridine-3-carboxylic acid), it suggests that nicotine contains a pyridine ring with some sort of group attached to it at the B- position. This group attached to pyridine ring is C5H10N and the oxidation

can be formulated as follows:

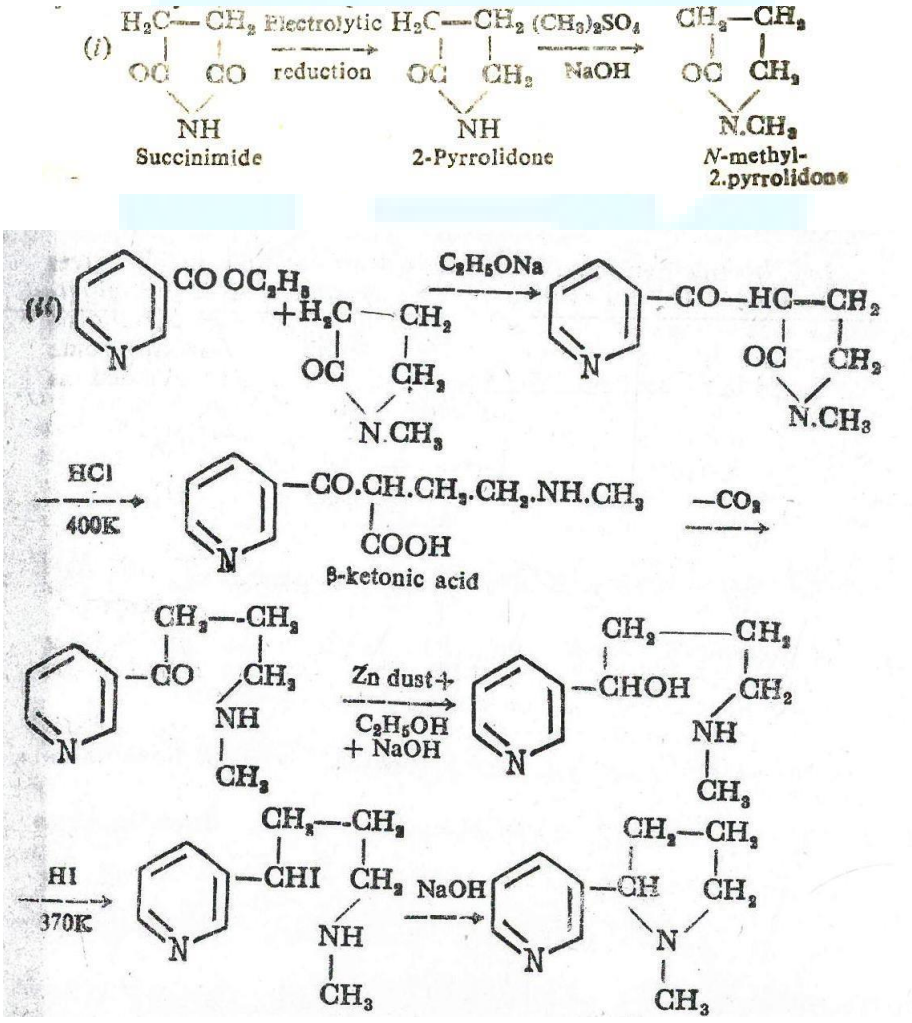
Nicotine hydriodide on treatment with methyl iodide gives a methiodide. This on oxidation yields hygrinic acid (N-methylpyr- rolidine-□ -carboxylic acid.

This indicates that pyridine ring has been destroyed during the above transformation and the group-$C_5H_{10}N$ attached to the pyridine ring in B-position is N-methylpyrrolidine.

Pyridine and pyrrolidine nuclei are joined through carbon atoms at B-position in pyridine and 2-position in pyrrolidine. This gives the structure of nicotine as:



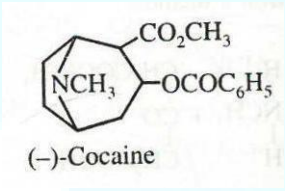
This formula has been further confirmed by its synthesis by Spath and Bpetshneider (1928):



The (+/-) - mixture of nicotine obtained by the above synthesis was resolved by forming salts with (+/-) -tartaric acid and (-) - nicotine thus obtained was found to be identical with the natural product.

COCAINE:

It was first isolated in 1860 from the leaves of *Erythroxylon coca L.,* (Coca Plant) which is mainly grown in South America, particularly in Peru and Bolivin and now grown in java and Ceylon. However, the plant from which cocaine is obtained (*i.e.* coca plant) should not be confused with *Theobrama cocoa,* the beans of which are source of cocoa and chocolate.



(–)-Cocaine

Isolation: In order to obtain the crude cocaine, the Peruvian leaves are powdered and thoroughly digested with lime or sodium carbonate and a little water. The digested solution is then extracted with light petroleum when the alkaloids get dissolved in the light petroleum layer. From the organic layer, the alkaloids are removed by shaking with a controlled amount of dilute sulphuric acid (avoiding excess). This acid solution when evaporated yields a crystalline precipitate of a larger portion of the cocaine which can be further purified by crystallization of its hydrocholoride.

Cocaine can also be extracted directly from the leaves with high boiling petroleum.

Properties:

1. It forms colourless crystals (m.p. 98^0C). It is sparingly soluble in water, but its hydrochloride is quite soluble. It is a strong tertiary base (pka 8.7).

2. The hydrochloride of cocaine is used as a local anaesthetic in eye surgery and dentistry. Usually, cocaine is injected along with adrenaline.

3. Cocaine is the habit forming drug and is, therefore, used with great care. Taken internally, it increases physical and mental power but the after-effects is deep depression.

Constitution:

1. ***Molecular Formula.*** From analytical data and molecular weight determination, it follows that the empirical and molecular formula of cocaine i.e., $C_{17}H_{21}NO_4$.

2. ***Nature of the Nitrogen Atom.*** It is a strong tertiary base (pka 8.7) and adds on one molecule of methyl iodide to form a methiodide. It also reacts with cyanogens bromide to give methyl bromide and cyanonorcocaine and thus contains a N-methyl group.

$$C_{17}H_{21}NO_4 + CH_3I \longrightarrow C_{17}H_{21}NO_4.CH_3I$$

1. ***Hydrolysis.*** When cocaine is heated with water, it is hydrolysed to methanol and benzoylecgonine.

$$C_{17}H_{21}NO_4 + H_2O \longrightarrow C_{16}H_{19}NO_4 + CH_3OH$$

Cocaine Benzoylecgonine

But benzoylecgonine contains a carboxyl group. Therefore, cocaine is the methyl ester of benzoylecgonine which is also proved by the fact that benzoylecgonine when heated with methyl alcohol in presence of hydrochloric acid yields cocaine.

When benzoylegonine is boiled with barium hydroxide solution. It undergoes further hydrolysis, yielding benzoic acid and ecognine.

$$C_{16}H_{19}NO_4 + H_2O \xrightarrow{Ba(OH)_2} C_9H_{15}NO_3 + C_6H_5COOH$$

Benzoylecgonine (-)-Ecgonine Benzoic acid

From the above reactions, it is evident that the constitution of cocaine depends on the constitution of ecgonine.

4. *Constitution of Ecgonine.* It is established as follows:

a) Its molecular formula is $C_9H_{15}NO_3$.

b) It is a tertiary base because it gives the crystalline additive compound $C_9H_{15}NO_3.CH_3I$ with methyl iodide. This reaction shows that ecgonine contains tertiary nitrogen atom.

c) As ecognine forms ester and salt with alcohol and alkali respectively, it means that it contains one *carboxyl group.*

d) The presence of - OH group is indicated by the fact that it reacts with acid chloride and anhydride to form acyl derivatives. Since this acyl derivative can be further esterified, it shows that ecgonine is both an alcohol and an acid.

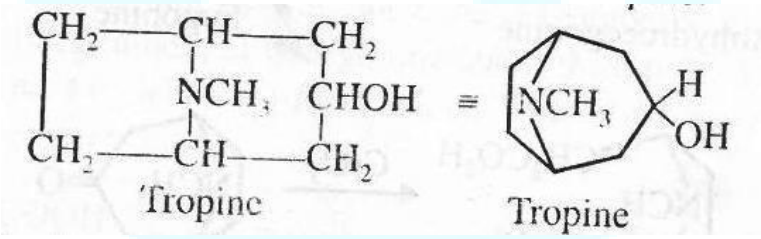
e) Ecgonine when oxidised with CrO_3 yields a ketone ecgoninone which soon loses a molecule of carbon dioxide to yield tropinone. The latter compound when further oxidised yields a mixture of tropinic acid and ecgoninic acid, former of which is also obtained from tropine.

$$C_9H_{15}NO_3 \xrightarrow{CrO_3} C_9H_{13}NO_3 \xrightarrow{-CO_2} C_8H_{13}NO \xrightarrow{CrO_3} C_8H_{13}NO_4 + C_7H_{11}NO_3$$

Ecgonine — Ecgoninone — Tropinone — Tropinic acid + Ecgoninic acid

From the nature of products obtained by oxidation of ecgonine, following conclusions are drawn :

(i) The reaction which involves the oxidation of ecgonine first to tropinone and then to tropinic acid reveals that ecgonine contains the tropane skeleton and furthermore position of the secondary alcoholic group in ecgonine remains the same as in tropine.

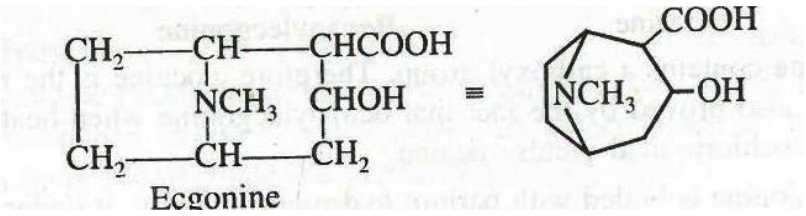


The close similarity between the structures of ecgonine and tropine is further proved by the fact that the dehydration of ecgonine yields anhydroecgonine which on decarboxylation yields tropidine. The latter compound is also formed by the dehydration of tropine.

$$\text{Ecgonine} \xrightarrow{-H_2O} \text{Anhydroecgonine} \xrightarrow{-CO_2} \text{Tropidine}$$

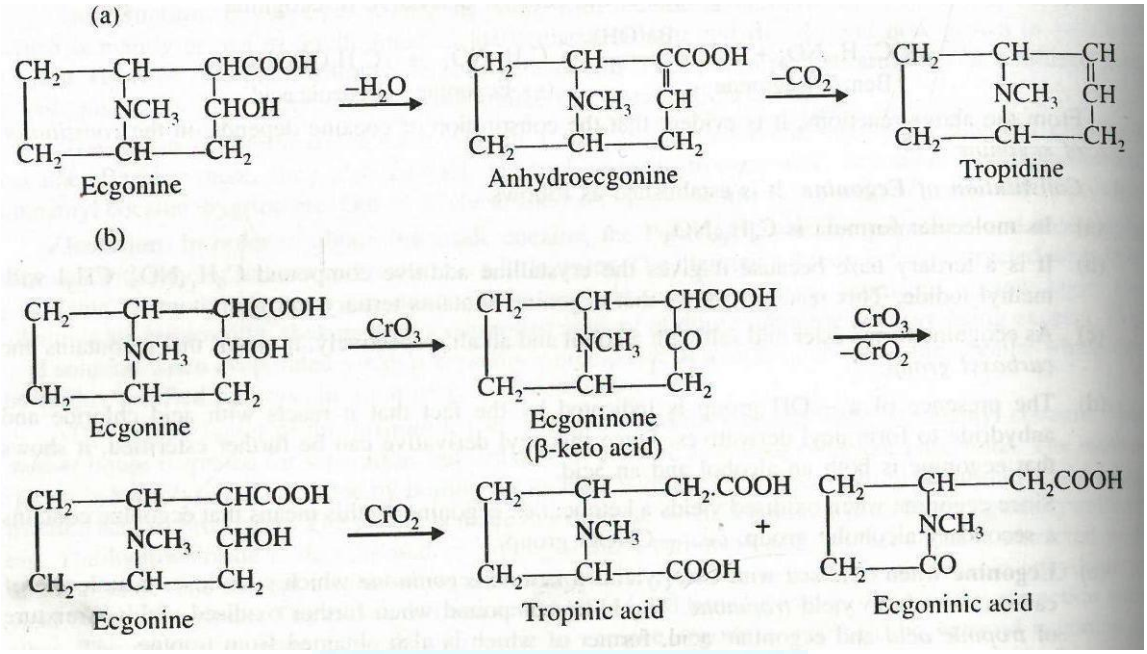
$$\text{Tropine} \xrightarrow{-H_2O} \text{Tropidine}$$

(ii) The easy decarboxylation of the ecogonine reveals that it is a B-keto acid. This interpretation is confirmed by the fact that Willstatter actually observed the formation of an unstable ketonic acid which lost carbon dioxide to yield tropinone. Thus, ecgonine is :



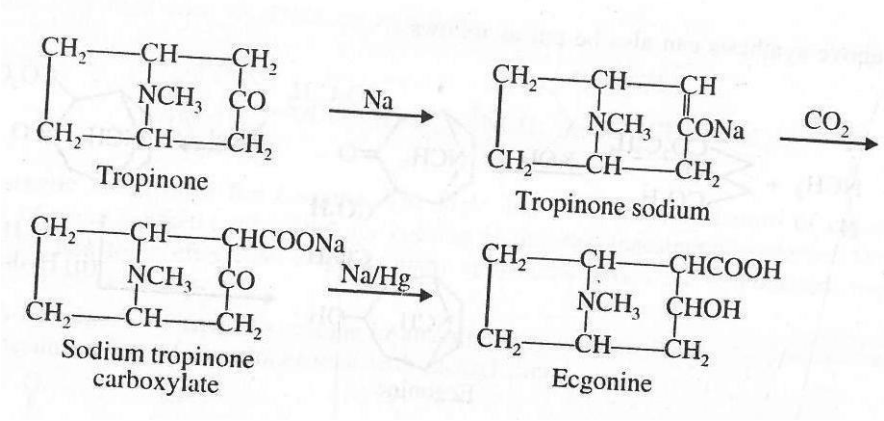
Ecgonine

f)The above structure of ecgonine explains all its reactions :



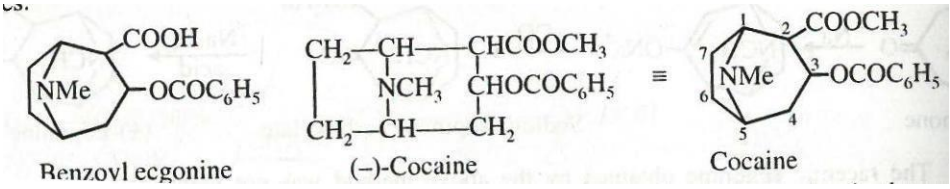
g) Finally the structure of ecgonine is proved by its synthesis:

The starting material is tropinone.

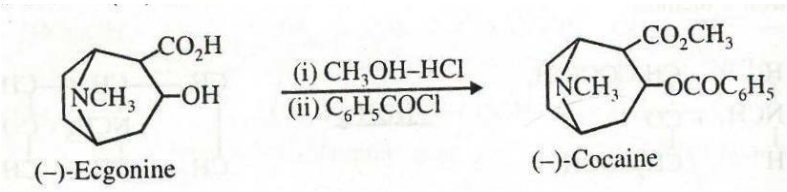


The racemic ecgonine obtained by the above method was not identical with (-) ecgonine obtained from (-). cocaine. However, its chemical properties were the same.

5. Constitution of Cocaine. Now since we know that cocaine is methyl ester of benzoyl ecgonine having a free carboxylic group; the benzoyl ecgonine and cocaine will be having the following structures.



The above structure of cocaine has been proved by its synthesis which consists in the resolution of the racemic ecgonine, esterification of (-). ecgonine followed by benzoylation to give cocaine identical to natural (-). form.



Similarly, (+) and (-) cocaines were obtained from the corresponding - ecgonines.

Carbohydrates

Carbohydrates (carbon hydrates, Cn(H2O)n, hence their name), are the most abundant class of organic compounds found in living organisms. They originate as products of photosynthesis: condensation of carbon dioxide requiring light, energy, and the pigment chlorophyll.

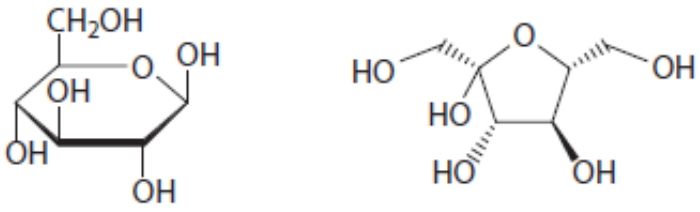
n CO2 + n H2O + energy → CnH2nOn + n O2

Carbohydrates, also known as polysaccharides or sugars, are a major source of metabolic energy, both for plants and for animals that depend on plants for food. They are a component of the energy transport compound ATP and are on recognition sites on cell surfaces. Importantly, sugars are also one of the three essential components of both DNA (e.g., ribose) and RNA (e.g., deoxyribose). The simplest carbohydrate is a monomer (monosaccharide), represented either
by a six- or five-membered ring structure, with the examples of glucose and ribose respectively.

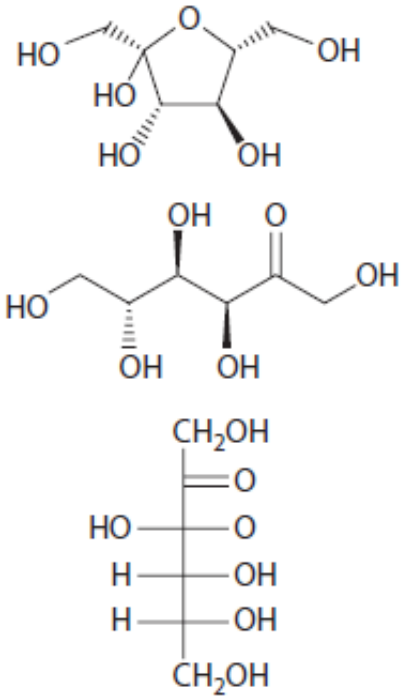
The six-membered ring sugar can be represented by several other forms designated by the structures. These forms of the monomer can be:
• As ketose sugars having a ketone function or an acetal equivalent
• As aldose sugars having an aldehyde function or an acetal equivalent

All compounds are isomers of glucose because the configurations of the OH group can vary on each of the carbons of the ring. The two rows represent common ways to draw the structures of the monomers.

When these monosaccharides are linked they form polysaccharide chains through glycosidic bond linkages. Linking the sugars can be in a linear or branched manner, or as a combination of both ways, and various carbohydrates (e.g., disaccharides, oligosaccharides, and polysaccharides) can be generated. Polysaccharides can attain very high molecular weight ranges of over 100,000 daltons.



Chemical structures of two monosaccharides: glucose and ribose.



Alternative forms of the six-membered ring sugar.

Glucose

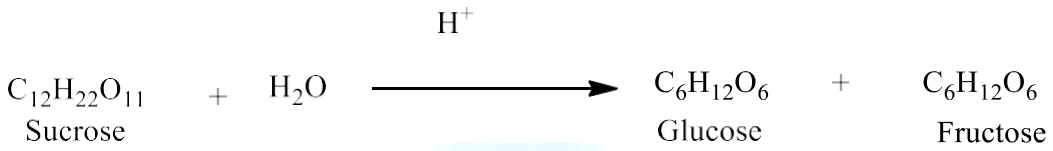
Glucose is most common monosaccharide. It is known as Dextrose because it occurs in nature principally as optically dextrorotatory isomer. Glucose is found in most sweet fruits, especially grapes (20-30%), and honey. It is an essential constituent of human blood. The blood normally conatains 65 to 110 mg (0.06 to 0.1%) of glucose per 100 ml. In diabetic persons the

level may be much higher. In combined form glucose occurs in abundance in cane sugar and polysaccharides such as starch and cellulose.

Preparation of Glucose

1. **From sucrose (Cane sugar)**

When sucrose in boiled with dilute HCl or H2SO4 in alcoholic solution, glucose and fructose are obtained in equal amounts.



2. *From Starch*

Glucose is produced commercially by the hydrolysis of starch by boiling it with dilute H2SO4 at high temperature under pressure.

in this process, an aqueous solution of starch obtained from corn is acidified with dilute H2SO4. It is then heated with high pressure steam in an autoclave. When the hydrolysis is complete, the liquid is neutralized with sodium carbonate to pH of 4-5. The resulting solution is concentrated under reduced pressure to get the crystals of glucose.

Physical properties of glucose

Some important physical properties of glucose are mentioned as under:

1. It is colourless sweet crystalline compound having m.p.419 K.
2. It is readily soluble in water, sparingly soluble in alcohol and insoluble in ether.
3. It forms a monohydrate having m.p. 391 K.
4. It is optically active and its solution is dextrorotatory. The specific rotation of fresh solution is + 112^0 C.
5. It is about three fourth as sweet as sugarcane i.e., sucrose.

Chemical properties of glucose

Chemical properties of glucose can be studied under the following headings:

***(A)** Reactions of aldehydic group*

1. **Oxidation**. (a) Glucose gets oxidized to gluconic acid with mild oxiding agents like bromine water

$$\underset{\text{Glucose}}{CH_2OH\,(CHOH)_4CHO} \xrightarrow[Br_2,\ H_2O]{[O]} \underset{\text{Gluconic acid}}{CH_2OH\,(CHOH)_4COOH}$$

Only-CHO group is affected.

(b) A strong oxidizing agent like nitric acid oxidizes both the terminal groups viz. –CH2OH and –CHO groups and saccharic acid or glucaric acid is obtained.

$$\underset{\text{Glucose}}{CH_2OH\,(CHOH)_4CHO} \xrightarrow[[O]]{HNO_3} \underset{\text{Glucaric acid}}{COOH\,(CHOH)_4COOH}$$

(d) Glucose gets oxidized to gluconic acid with ammonical silver nitrate (Tollen's reagent) and alkaline copper sulphate (Fehling solution). Tollen's reagent is reduced to metallic silver (silver mirror) and Fehling solution to cuprous oxide which is a red precipitate.

(i) With Tollen's reagent

$$AgNO_3 + NH_4OH \longrightarrow AgOH + NH_4NO_3$$

$$2AgOH \longrightarrow Ag_2O + H_2O$$

$$\underset{\text{Glucose}}{CH_2OH(CHOH)_4CHO} + Ag_2O \longrightarrow \underset{\text{Gluconic acid}}{CH_2OH(CHOH)_4COOH} + \underset{\text{Silver mirror}}{2Ag\downarrow}$$

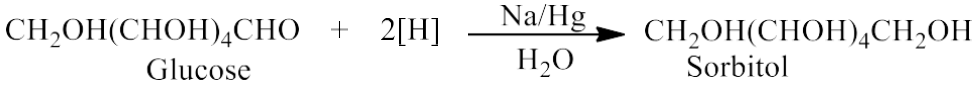
(ii) With Fehling solution

$$CuSO_4 + 2NaOH \longrightarrow Cu(OH)_2 + Na_2SO_4$$

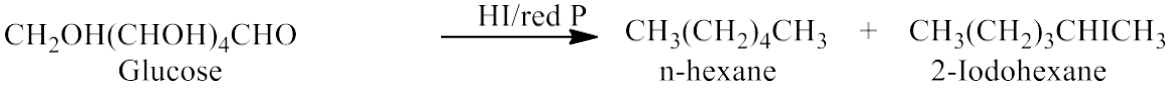
$$Cu(OH)_2 \longrightarrow CuO + H_2O$$

$$\underset{\text{Glucose}}{CH_2OH(CHOH)_4CHO} + 2CuO \longrightarrow \underset{\text{Gluconic acid}}{CH_2OH(CHOH)_4COOH} + \underset{\text{Red ppt.}}{Cu_2O}$$

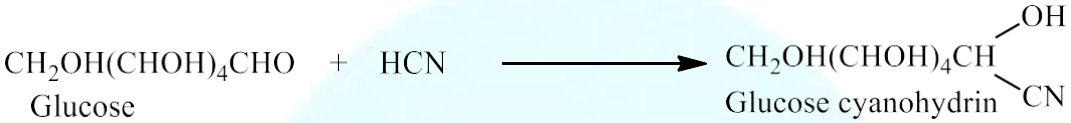
2. **Reduction** (a) glucose is reduced to sorbitol or Glucitol on treatment with sodium amalgam and water.



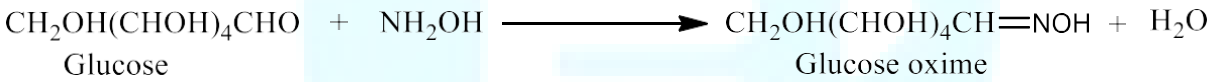
(b) On reduction with conc. HI and red P at 373 K glucose gives a mixture of n-hexane and 2-idohexane



3. **Reaction with HCN**. Like aldehydes, glucose reacts with HCN forming cyanohydrins.

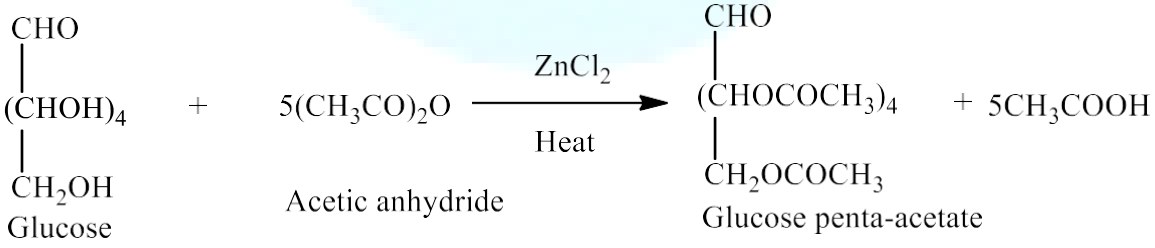


4. **Reaction with hydroxylamine**. Glucose forms glucose oxime.

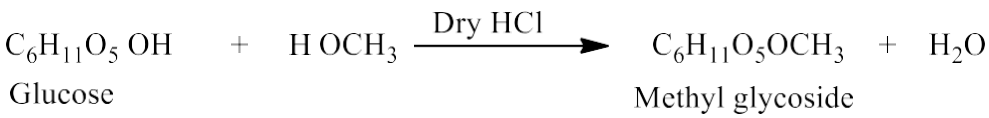


(B) *Reactions of hydroxyl groups*

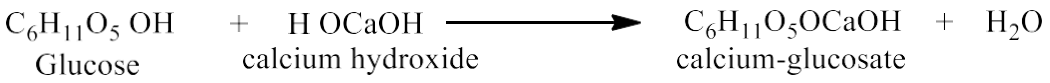
1. **Reaction with acetic anhydride or acetyl chloride**. Glucose forms penta acetate with acetic anhydride of acetyl chloride.



2. **Reaction with methyl alcohol**. Glucose reacts with methy alcohol in the presence of dry HCl gas to form methyl glucoside.

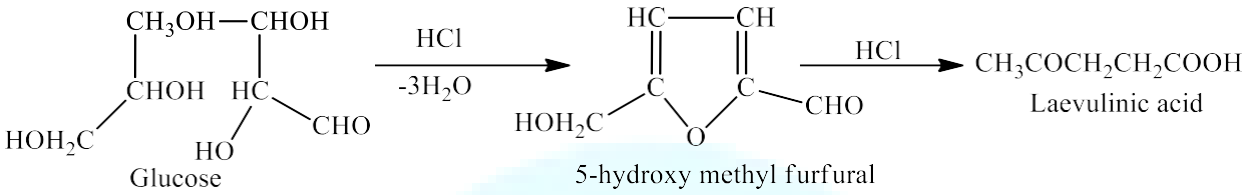


3. **Reaction with metallic hydroxides**. Glucose reacts with calcium hydroxide to form calcium glucosate which is water soluble.

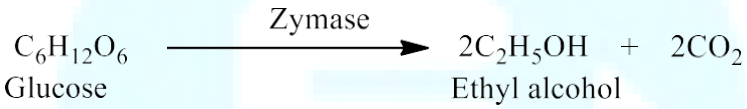


(C) *Miscellaneous reactions*

1. Action of acids. On warming with conc.HCl, glucose forms 5-hydroxy methyl furfural, which on further reaction gives laevulinic acid.

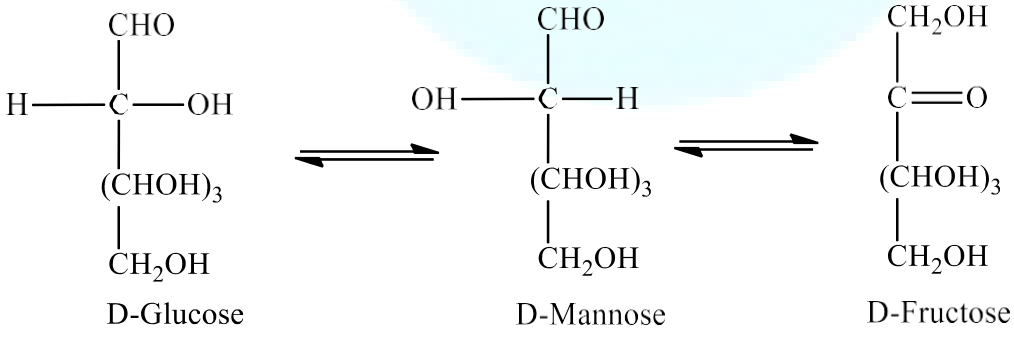


2. Fermentation. Glucose undergoes fermentation into ethyl alcohol in the presence of the enzyme zymase.

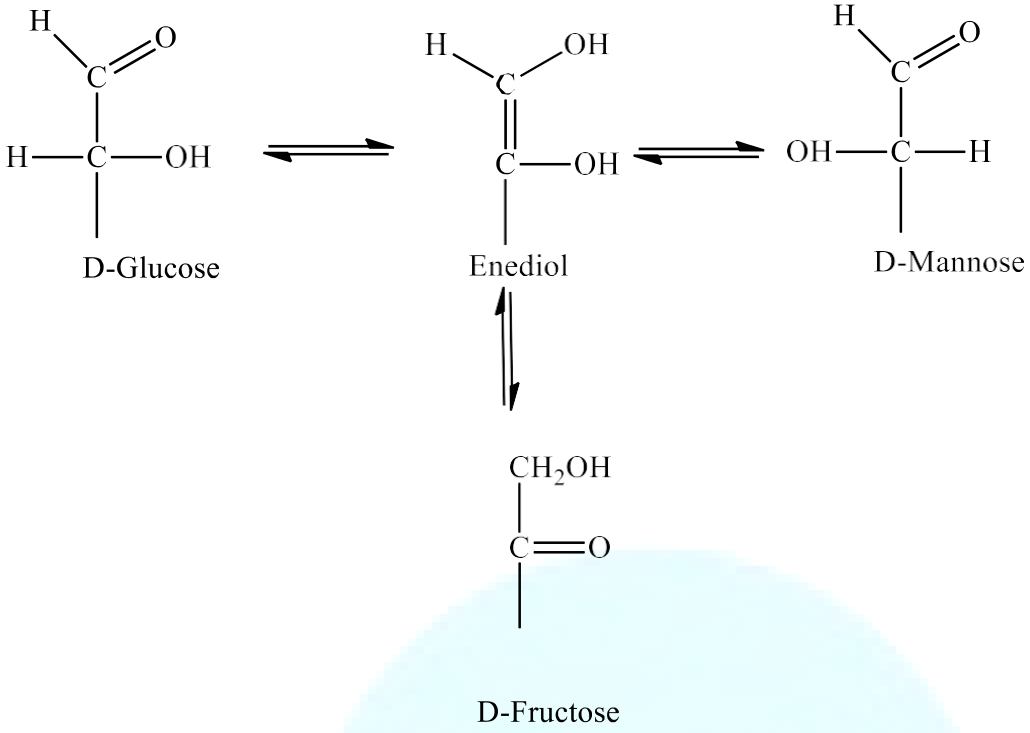


This reaction called alcoholic fermentation is the basis of manufacture of wines and alcohol.

3. Reaction with Alkalies. When warmed with strong sodium hydroxide solution, glucose forms a brown resinous product. In dilute alkali solution, D-glucose rearranges to give a mixture of D- glucose, D-mannose and d-fructose.



The above equilibrium is established via the enediol starting from any of these three hexoses.



That is why D-Fructose, although it has a ketonic C=O group, reduces Fehling's solution or Tollen's reagent. The rearrangement reaction of a monosaccharides in weakly alkaline solutions to give a mixture of isomeric sugars, is named as Lobry de Bruyn Van Ekestein rearrangement.

Structure of glucose

1. On the basis of elemental analysis and molecular weight determination the molecular formula of glucose is $C_6H_{12}O_6$.
2. The reduction of glucose with red phosphorus and HI gives n-hexane.

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \xrightarrow{HI/redP} \underset{\text{n-hexane}}{CH_3CH_2CH_2CH_2CH_2CH_3}$$

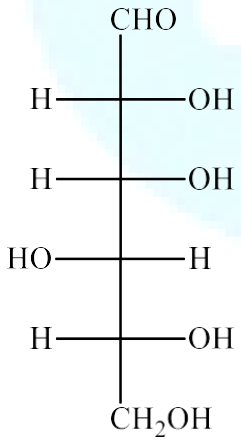
Therefore, the six carbon atoms of glucose form a straight chain.

3. It forms penta acetate on treatment with acetic anhydride which indicates the presence of five hydroxyl groups in the molecule.
4. Glucose reacts with hydroxyl amine to form an oxime and with hydrogen cyanide to form cyanohydrins. It indicates the presence of a carbonyl group. It also forms phenylhydrazone on treatment with phenylhydrazine.

5. The mild oxidation of glucose with bromine water or sodium hypobromide yields a monocarboxylic acid (gluconic acid) containing same number of carbon atoms as in glucose, i.e., six. This indicates that the carbonyl group must be aldehyde group.
6. The catalytic reduction of glucose gives a hexahydric alcohol (sorbitol) which gives hexaacetate on treatment with acetic anhydride. The sixth hydroxyl group must be obtained by the reduction of aldehyde group, thus further confirming the presence of an aldehyde group and five hydroxyl groups in glucose.
7. Oxidation of gluconic acid with nitric acid yields a dicarboxylic acid (glucaric acid) with the same number of carbon atoms as in glucose. Thus besides aldehyde group, glucose must contain a primary alcoholic group also, which generates the second carboxylic group on oxidation.
8. Glucose is a stable compound and does not undergo dehydration easily, indiacating that not more than one hydroxyl group is bonded to a single carbon atom. Thus all the hydroxyl groups are attached to different carbon atoms.

Open –chain structure of glucose

On the basis of above reactions, Fisher assigned an open chain structure of glucose shown below as structure I



I

D-(+)-Glucose

The above structure of glucose is also confirmed by the cleavage reaction of glucose with periodic acid. Five moles of periodic acid are consumed by one mole of glucose giving five moles of formic

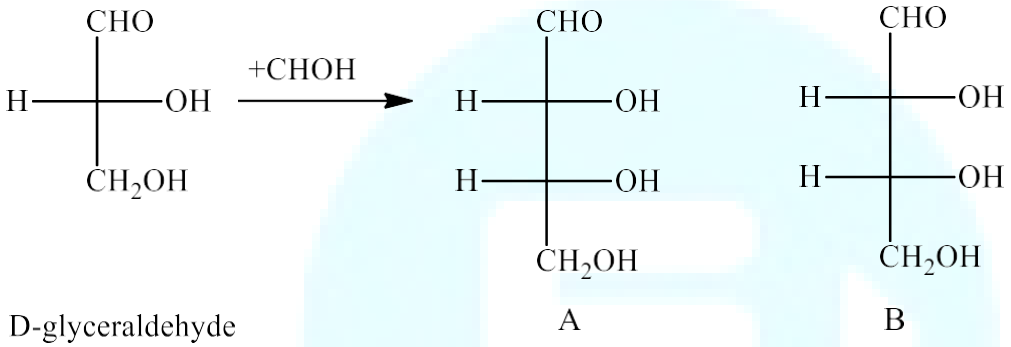
acid and one mole of formaldehyde.

$$\underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Periodic acid}}{5HIO_4} \longrightarrow HCHO + 5HCOOH + 5HIO_3$$

Configuration of D-Glucose

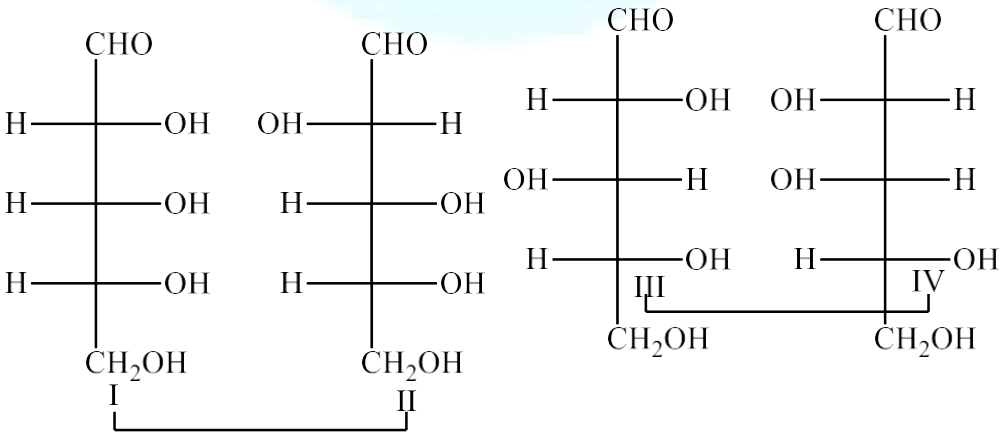
The configuration of D-glucose was proved by Emil Fisher by arguments similar to the ones stated below.

1. Construction of four possible D-pentoses. Taking the configuration of D-glyceraldehyde as the standard, two possible D-aldotetroses (A and B) may be constructed by adding a CHOH just below CHO, placing OH to the right and then to the left.



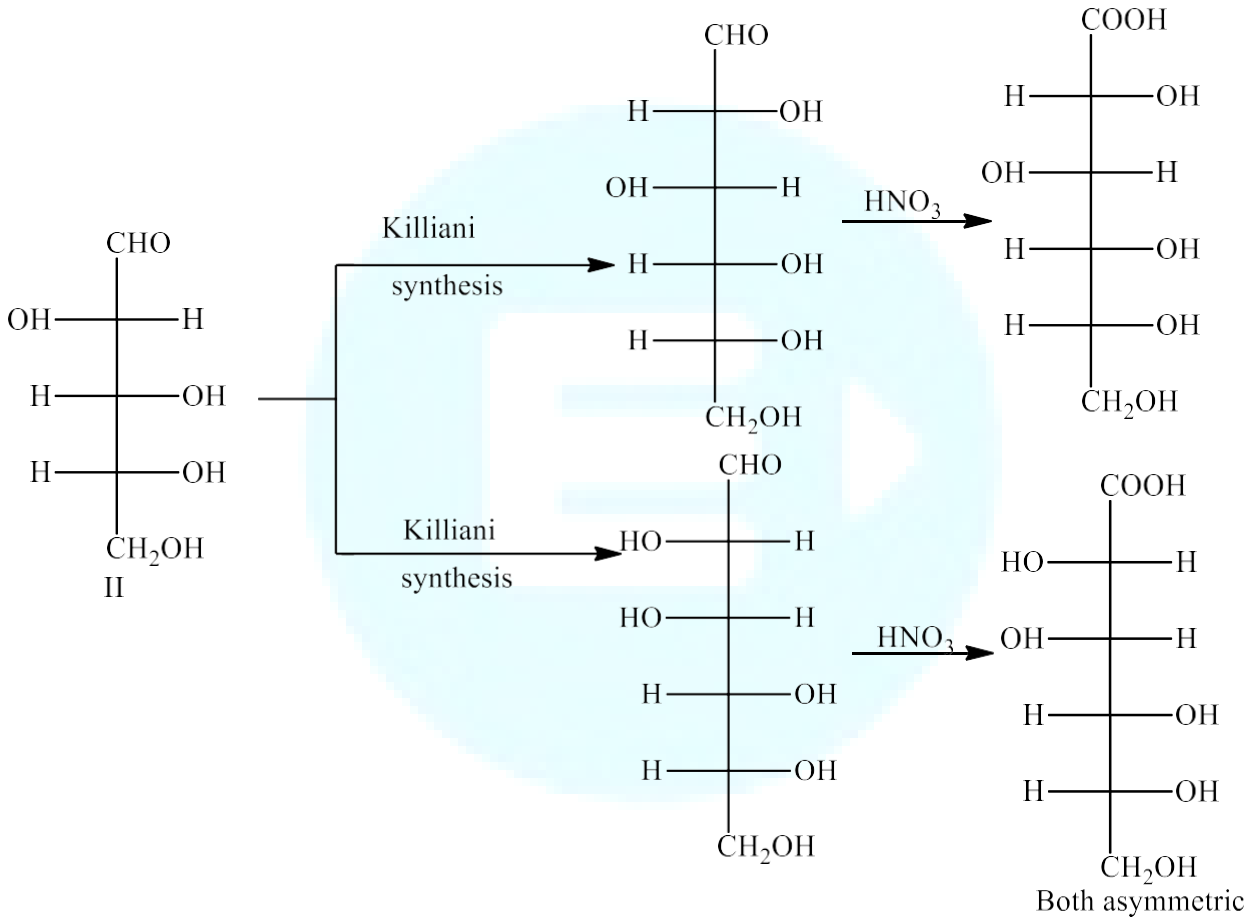
D-glyceraldehyde A B

Similarily, each of the two D-tetroses (A and B) gives two D-aldopentoses. Thus four possible D-aldopentoses are:



From A From B

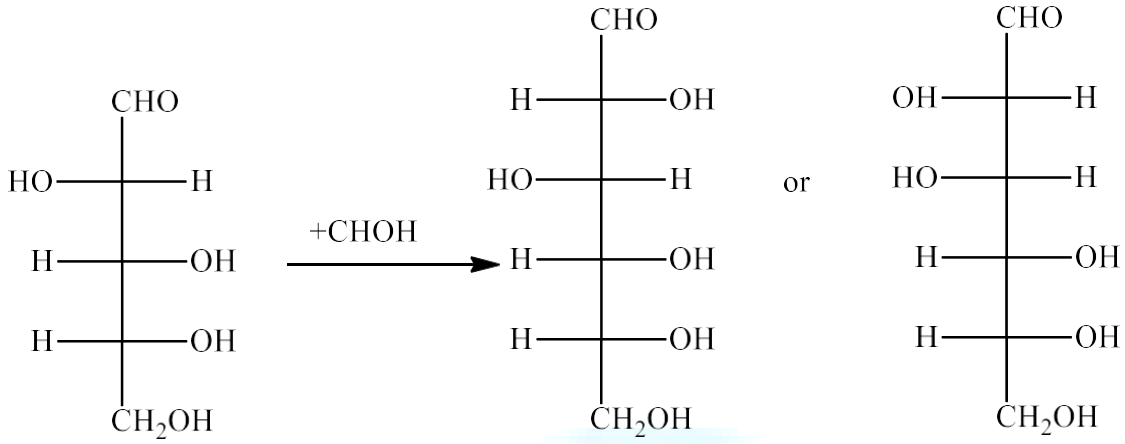
2. D-Arabinose has configuration II or IV. Oxidation of D-arabinose with nitric acid oxidizes the terminal CHO and CH_2OH groups yielding two optically active dicarboxylic acids. The forms II and IV can form two optically active diacids, while I and III can give meso acids only that have a plane of symmetry, therefore, D-arabinose is either II or IV.
3. Configyration II confirmed for D-arabinose. D-arabinose by Killiani-Fisher synthesis yields two epimeric aldohexoses, D-glucose and D-mannose. Thses of oxidation with nitric acid form two optically active dicarboxylic acids. This is theoretically possible onlyif D-arabinose has the configuration II and not IV.



Proceeding similarily, you will find that if D-arabinose had configuration IV, of the two dicarboxylic acids derived from it, one would be meso and one asymmetric. Hence D-arabinose has the configuration II.

4. Ruff degradation of D-glucose and D-mannose produces D-arabinose in each case. In ruff degradation the CHOH below CHO is destroyed. Therefore , the configuration of the two

aldohexoses, D-glucose and D-mannose, can be derived by adding a new CHOH below CHO in form II of D-arabinose.

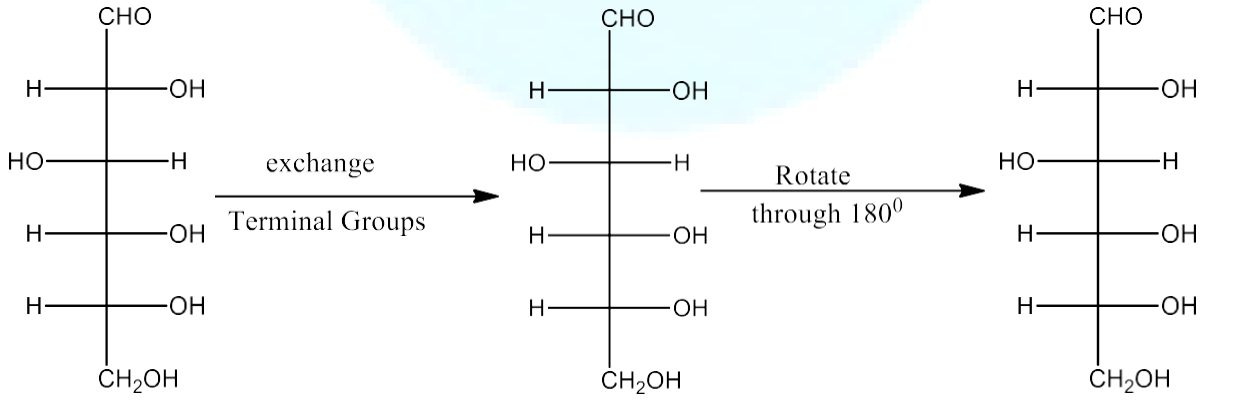


D-arabinose

II V VI

Hence D-glucose has configuration V or VI.

5. D-Glucose and L-Glucose yield the same dicarboxylic acid. This means thattwo sugars differ only in respect of the position of the terminal groups (CHO and CH_2OH). Therefore , the exchange of the terminal groups in D-glucose should be able to give a different aldohexose (L-glucose). Let us now examine configuration formula V and VI (one of which is D-glucose) from the angle.



V VI VII

Different from V

If VII is rotated through 180^0 in the plane of paper, it gives an aldohexose VII, different from V.

a similar procedure with formula VI does not give rise to a different sugar.

From the above arguments it is evident that D-glucose has the configuration as shown by the form V.

Cyclic structure of D-Glucose

The open chain structure of glucose explained most of its properties. However, it could not explain the following facts.

1. Despite having an aldehyde group, glucose does not undergo certain characteristic reactions of aldehyde,

(a) Glucose does not react with sodium bisulphate to form addition product.

(b) Glucose does not react with ammonia.

(c) Glucose does not give Schiff's test and 2, 4-DNP test like other aldehydes.

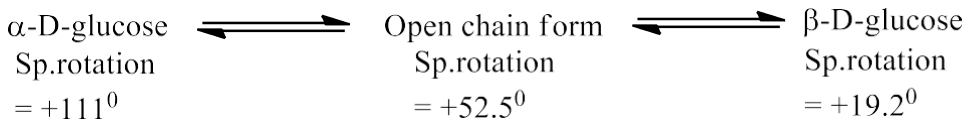
2. Glucose reacts with hydroxylamine to form an oxime but glucose pentaacetate does not react with hydroxylamine. This shows that –CHO group is not present in glucose pentaacetate.
3. **D (+)-Glucose exist in two stereoisomeric forms i.e., α- D (+)-Glucose and β- D (+)-Glucose.** These two forms are crystalline and have different m.p and optical rotations. When glucose was crystallized from a concentrated solution at 303 K, it gave α-form of glucose having m.p 419 K and $[\alpha]_D = +111^0$. On the other hand, the β-form of glucose is obtained on crystallization of glucose from a hot saturated solution of at a temperature above 371 K. The β-form of glucose has m.p 423 K and $[\alpha]_D = +19.2^0$.
4. **Mutarotation**. When either of two forms of glucose (α- D-glucose and β- D-glucose) are dissolved in water and allowed to stand, these get slowly converted into other form and a equilibrium mixture of both α- D-glucose (36 %) and β- D-glucose (about 64%) is formed.

The formation of equilibrium mixture can be explained as:

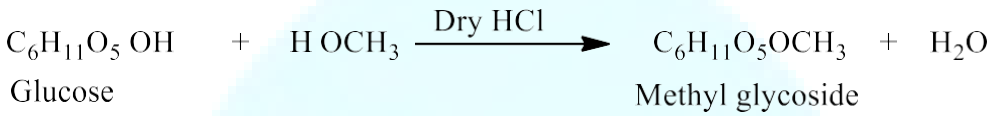
The α- D-glucose has a specific rotation of $+111^0$, while β- D-glucose has a specific rotation of $+19.2^0$. When α-form is dissolved in water, its specific rotation falls until a constant value of $+52.5^0$ is reached. On the other hand, when β-form is dissolved in water, its specific rotation increases and becomes constant at 52.5^0.

This spontaneous change in specific rotation of an optically active compound with time to an equilibrium value is called mutarotation.(Latin, muto means to change).

Thus, there is an equilibrium mixture of α- and β-forms in the solution



5. Glucose forms isomeric methyl glucosides. When glucose is heated with methanol in the presence of dry HCl, it gives two isomeric monomethyl derivatives known as α-D-glucoside (m.p. = 438 K) and β-D-glucoside (m.p. 380 K).



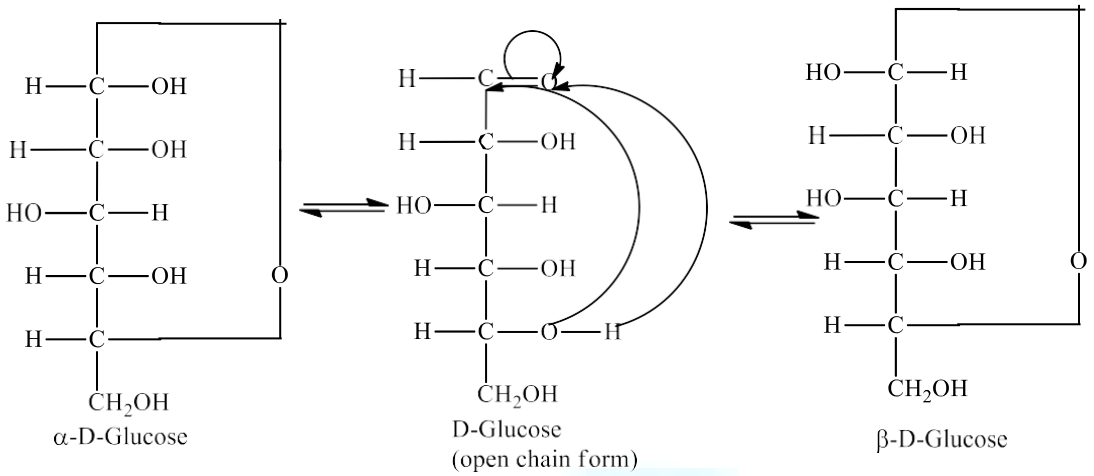
These two glucosides do not reduce Fehling's solution and also do not react with HCN or NH2OH indicating that the free –CHO group is not present but it is converted to –COOH group.

Cyclic structure of Glucose

Anomers:

Glucose forms a hemiacetal between the –CHO group and the -OH group on the C_5 atom. As a result, of cyclisation, C_1 becomes asymmetric (chiral) and the newly formed –OH group may be either on the left or on the right in Fisher projection formulae. These results in the formation of two isomerswhich differ in the orientation of H and –OH groups around C1 atom. These isomers are known as α- D-glucose and β- D-glucose. The isomer having the –OH group on the right is called α- D-glucose and one having the –OH group on the left is called β- D-glucose. Such pairs of optical isomers which differ in the configuration only around C_1 atom are called anomers.

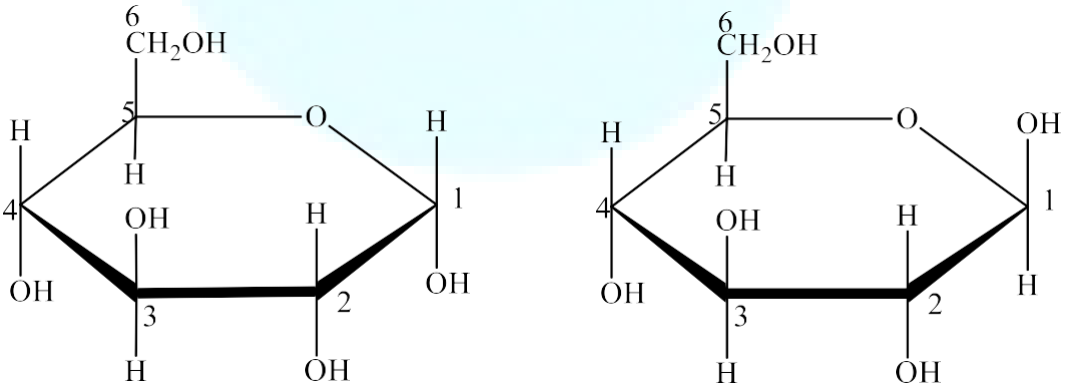
These two forms are not mirror image of each other, hence are not enantiomers. The C1 carbon is known as anomeric carbon or glycosidic carbon.



The above representations are called Fisher projection formulae.

Haworth projection formulae or pyranose structures of D-Glucose.

In Haworth structures drawn with the heterocyclic oxygen in the upper right corner, the α-form has the –OH group on C1 pointing "down". The β-form has the same group pointing "up". For D-sugars, the free –CH2OH group of an aldohexose is drawn above the plane of ring when ring oxygen is in the upper right. The rest is the simple, the groups on the left of the Fisher projection are up and those on the right are down in the Haworth structure.



α-D-Glucose

or

α-D-Glucopyranose

β–D-Glucose

or

β-D-Glucopyranose

Fructose

Fructose is another commonly known monosaccharide having the same molecular formula as glucose. It is laevorotatory because it roatates plane polarized light towards the left. It is present abundantly in fruits. That is why it is called fruit-sugar also.

Physical properties

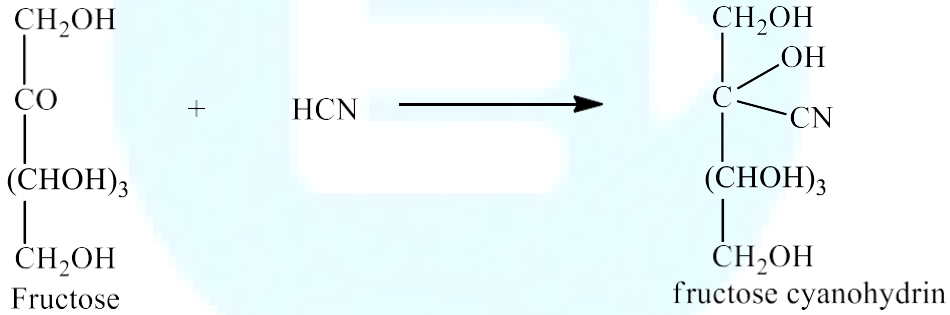
1. It is sweetest of all known sugars.
2. It is readily soluble in water, sparingly soluble in alcohol and insoluble in ether.
3. It is white crystalline solid with m.p. 375 K.
4. Fresh solution of fructose has a specific rotation -133^0.

Chemical properties of fructose

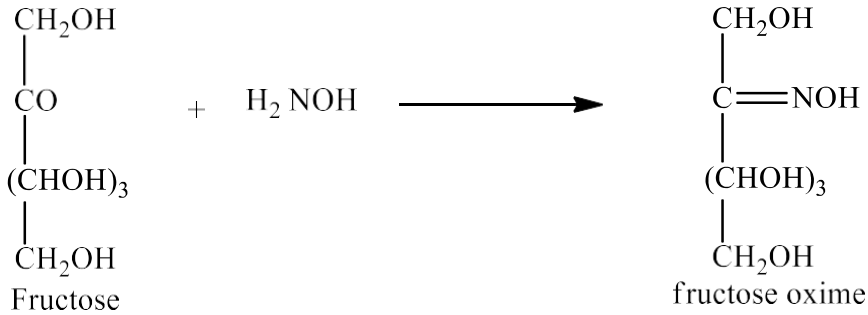
Chemical properties of fructose can be studied under the following heads:

(A) Reactions due to ketonic group

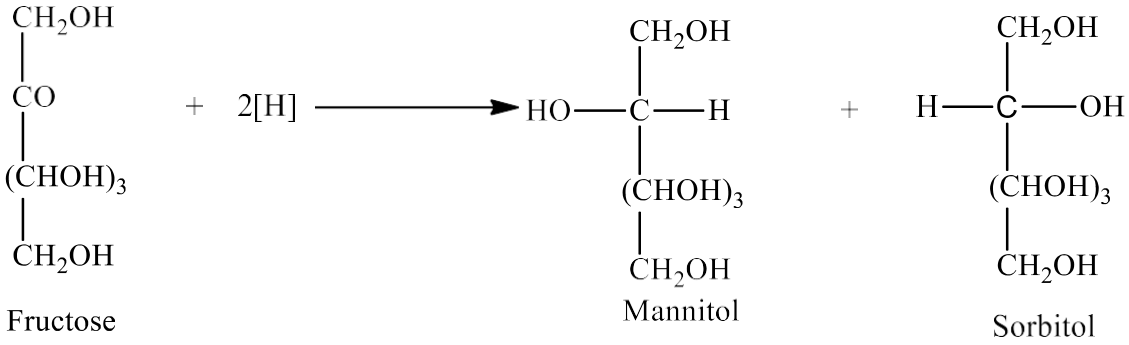
1. **Reaction with HCN**. Fructose reacts with HCN to form cyanohydrins.



2. **Reaction with hydroxylamine**. Fructose reacts with hydroxylamine to form an oxime.

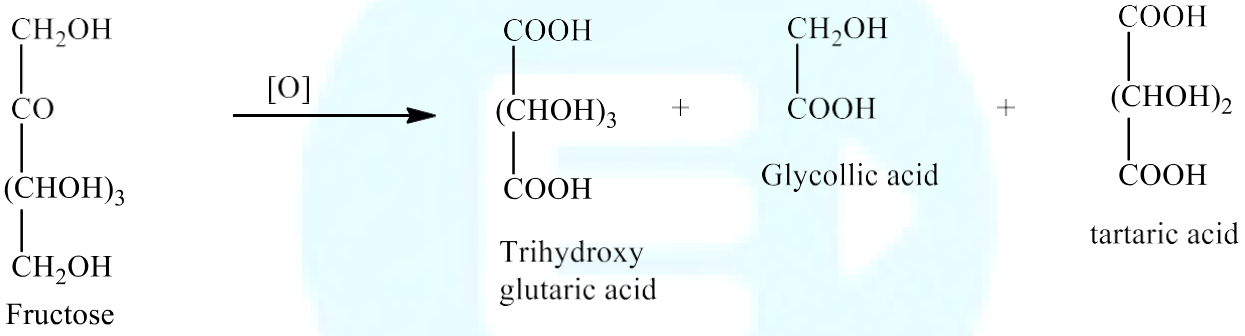


3. **Reduction**. Fructose gives a mixture of sorbitol and mannitol on reduction with Na-Hg and water or catalytic hydrogenation.



4. **Oxidation.** (i) there is no action of mild oxidizing agent like bromine water on fructose.

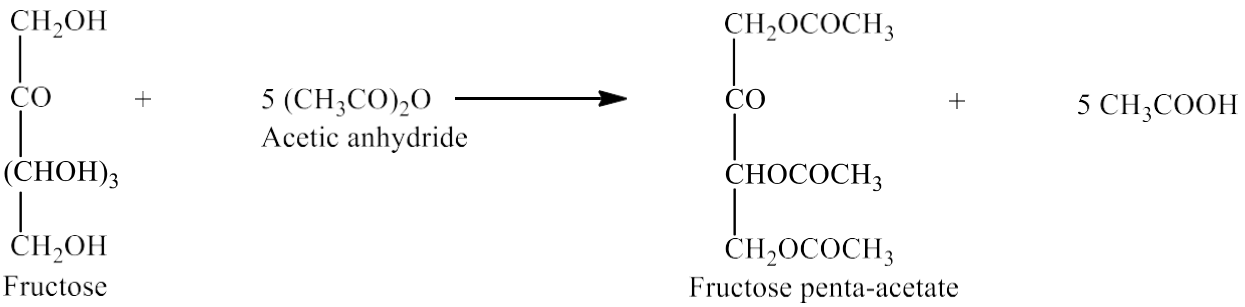
   (ii) Strong oxidizing agents like nitric acid oxidize fructose into a mixture of trihydroxy glutaric, glycolic and tartaric acids.



   (iii) Unlike other ketones, it reduces Tollen's reagent and Fehling solution. This is due to the presence of traces of glucose in alkaline medium.

[B] reactions of the alcoholic group

1. Acetylation . with acetic anhydride or acetyl chloride, fructose forms penta-acetate.



2. Reaction with methyl alcohol (glucoside formation). Fructose reacts with methyl alcohol in the presence of dry HCl gas forming methyl fructoside.

$$\underset{\text{Fructose}}{C_6H_{11}O_5OH} + HOCH_3 \xrightarrow{\text{Dry HCl}} \underset{\text{Methyl fructoside}}{C_6H_{11}O_5OCH_3} + H_2O$$

3. *Reaction with metallic hydroxides (fructosate formation)*

$$\underset{\text{Fructose}}{C_6H_{11}O_5OH} + \underset{\text{calcium hydroxide}}{HOCaOH} \longrightarrow \underset{\text{calcium-fructosate}}{C_6H_{11}O_5OCaOH} + H_2O$$

Structure of Fructose

1. elemental analysis and molecular weight determination of fructose show that it ahs the molecular formula C6H12O6.

2. fructose on reduction gives sorbitol which on reduction with HI and red P gives a mixture of n-hexane and 2-Iodohexane. This reaction indicates that six carbon atoms in fructose are in a straight chain.

3. Fructose reacts with hydroxylamine, HCN and phenylhydrazine. It shows the presence of _CHO or C=O group in the molecule of fructose.

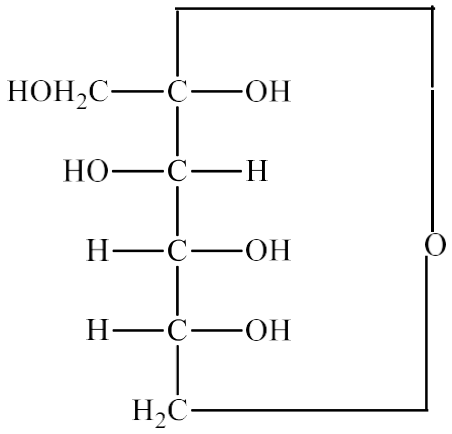
4. On treatment with bromine water , no reaction takes palce. This rules out the possibility of presence of –CHO group.

5. on oxidation with nitric acid, it gives glycollic acid and tartaric acids which contain smaller number of carbon atoms than fructose. This shows that a ketonic group is present at position 2. It is at this point that the molecule is broken.

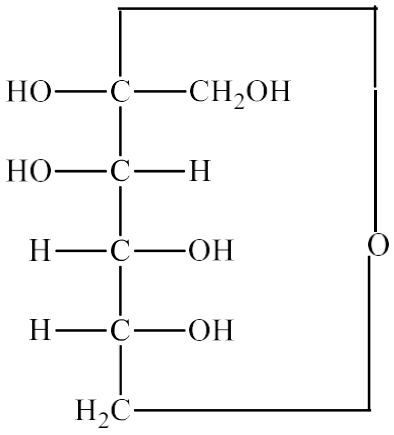
Cyclic structure of D-Fructose

Fructose shows the property of mutarotation. This means that it exists in two forms α-fructose and β-fructose which are cyclic in structure and change into each other via the open chain structure. The cyclic and pyranose structures of α-D-fructose and β-D-fructose are represented

below:

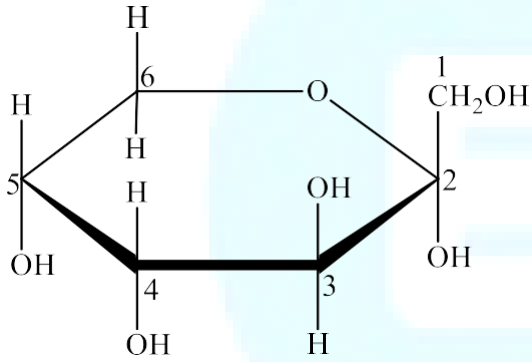


α-D-fructose

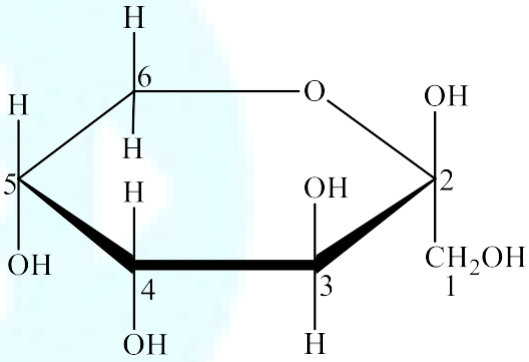


β-D-fructose

Haworth Pyranose structure

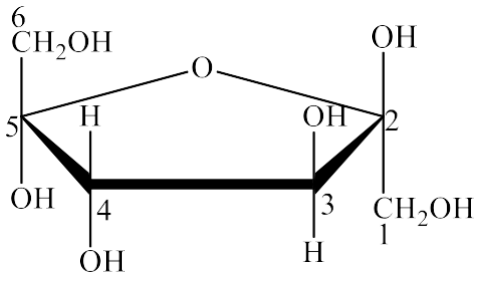


α-D-fructopyranose



β-D-fructopyranose

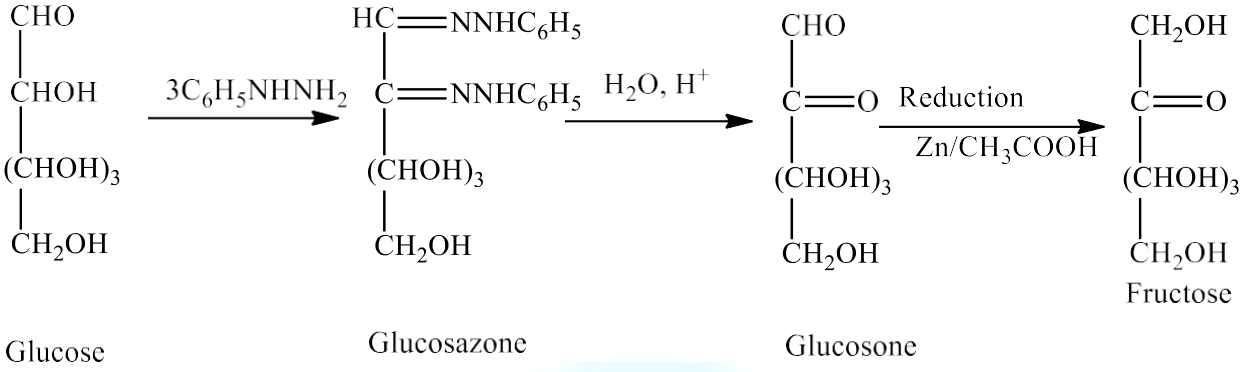
However, when fructose is linked to glucose in a sucrose molecule, it has the furanose structure as shown below:



β-D-fructofuranose

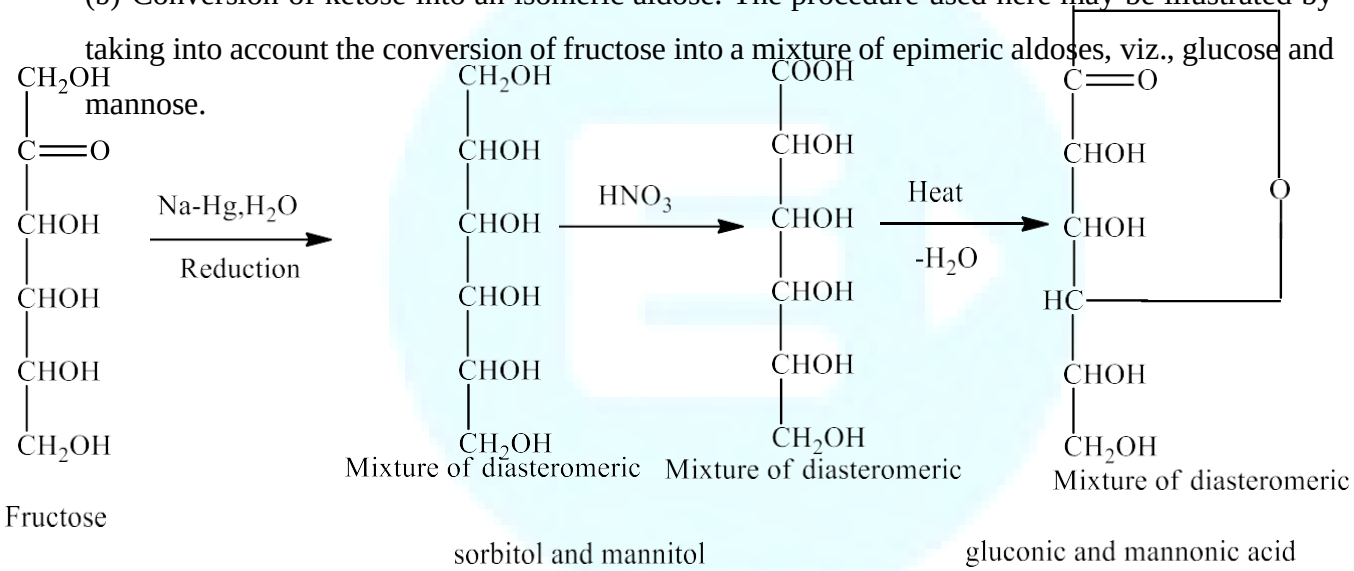
INTERCONVERSION OF GLUCOSE AND FRUCTOSE

(a) Conversion of an aldose into an isomeric ketose. The procedure used for this purpose may be illustrated by taking into account the conversion of glucose into fructose.

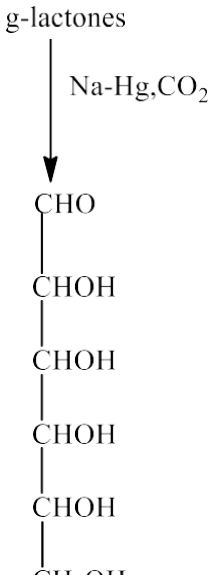


Glucose Glucosazone Glucosone

(b) Conversion of ketose into an isomeric aldose. The procedure used here may be illustrated by taking into account the conversion of fructose into a mixture of epimeric aldoses, viz., glucose and mannose.



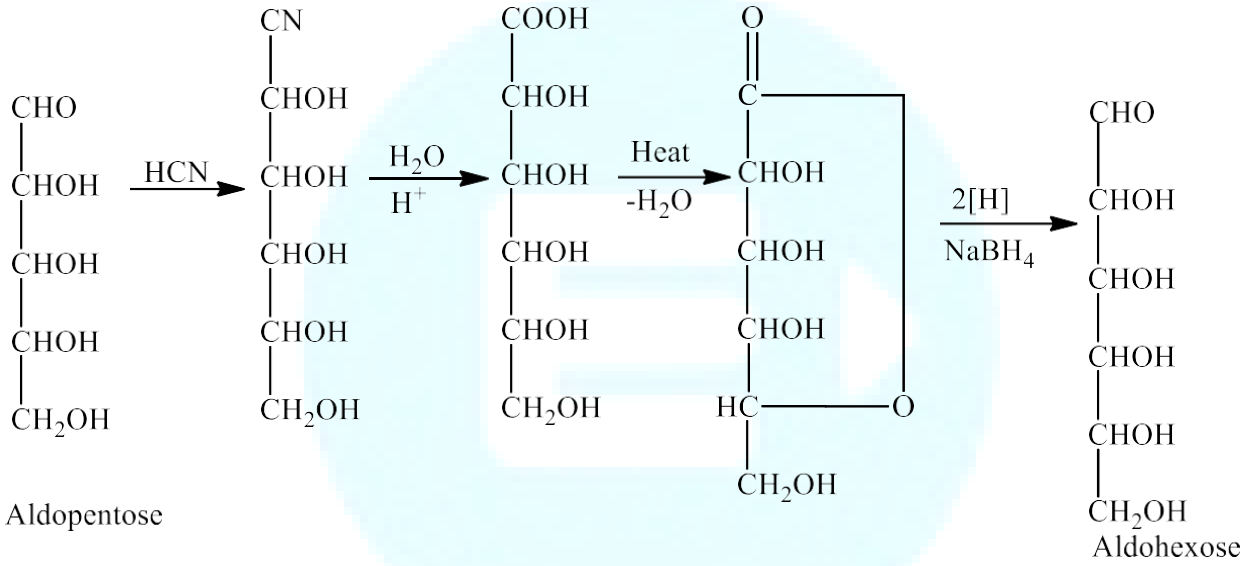
Fructose Mixture of diasteromeric sorbitol and mannitol Mixture of diasteromeric gluconic and mannonic acid



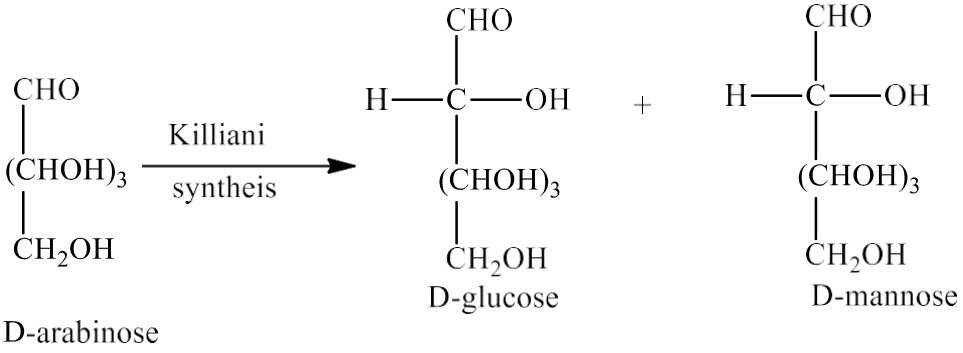
C HAIN LENGTHENING AND CHAIN SHORTENING OFALDOSE

(a) *Lengthening of aldoses: Killiani-Fisher synthesis*

The aldose chains may be lengthened by one carbon atom by a procedure known as **Killiani-Fisher synthesis.** Thus an aldose may be converted to the next higher member by the following steps: (1) Formation of cyanohydrins; (2) hydrolysis of –CN to –COOH, giving aldonic acid; (3) conversion of aldonic acid to lactone by heating; (4) reduction of lactone with sodium borohydride, NaBH4, to get higher aldose. For illustration, the overall change is the creation of an asymmetric centre at C-2 where a new CHOH has been added. Therefore their result two aldoses with one carbon more and differing only in configuration at C-2.



Taking a specific example, D-arabinose by killiani-Fisher synthesis gives two isomeric aldohexoses, D-glucose and D-mannose which differ only in the configuration at C-2



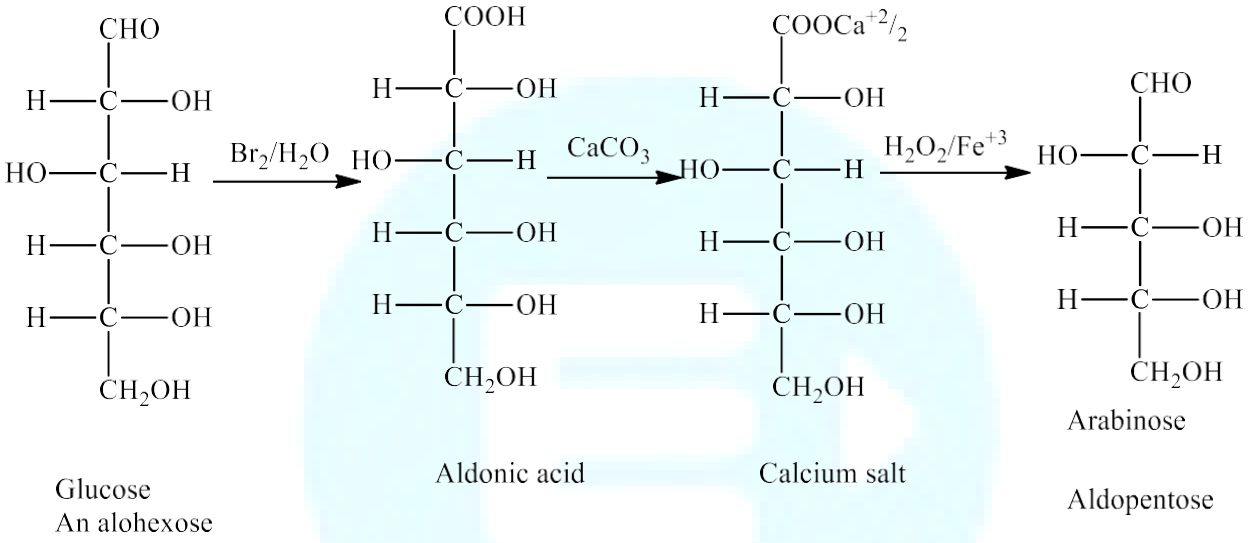
Such sugars which differ in configuration only at one asymmetric centre (C-2) are called

Epimers.

(b) *Shortening of aldoses*

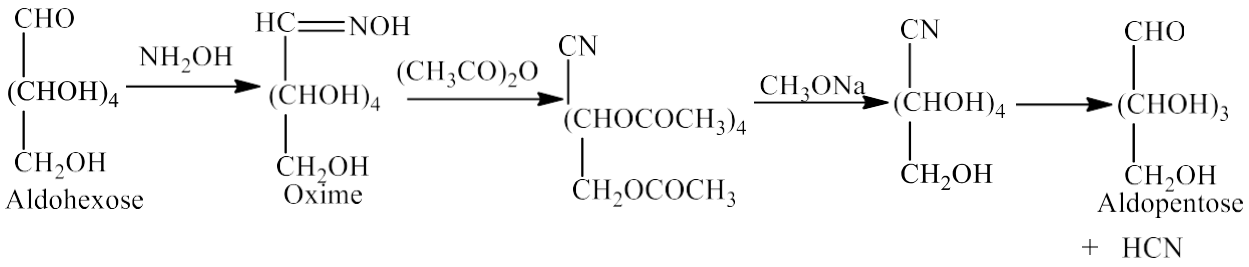
(1) Ruff degradation. An aldose may be converted into a lower aldose having one carbon atom less, i.e., the carbon chain may be shortened by Ruff degradation.

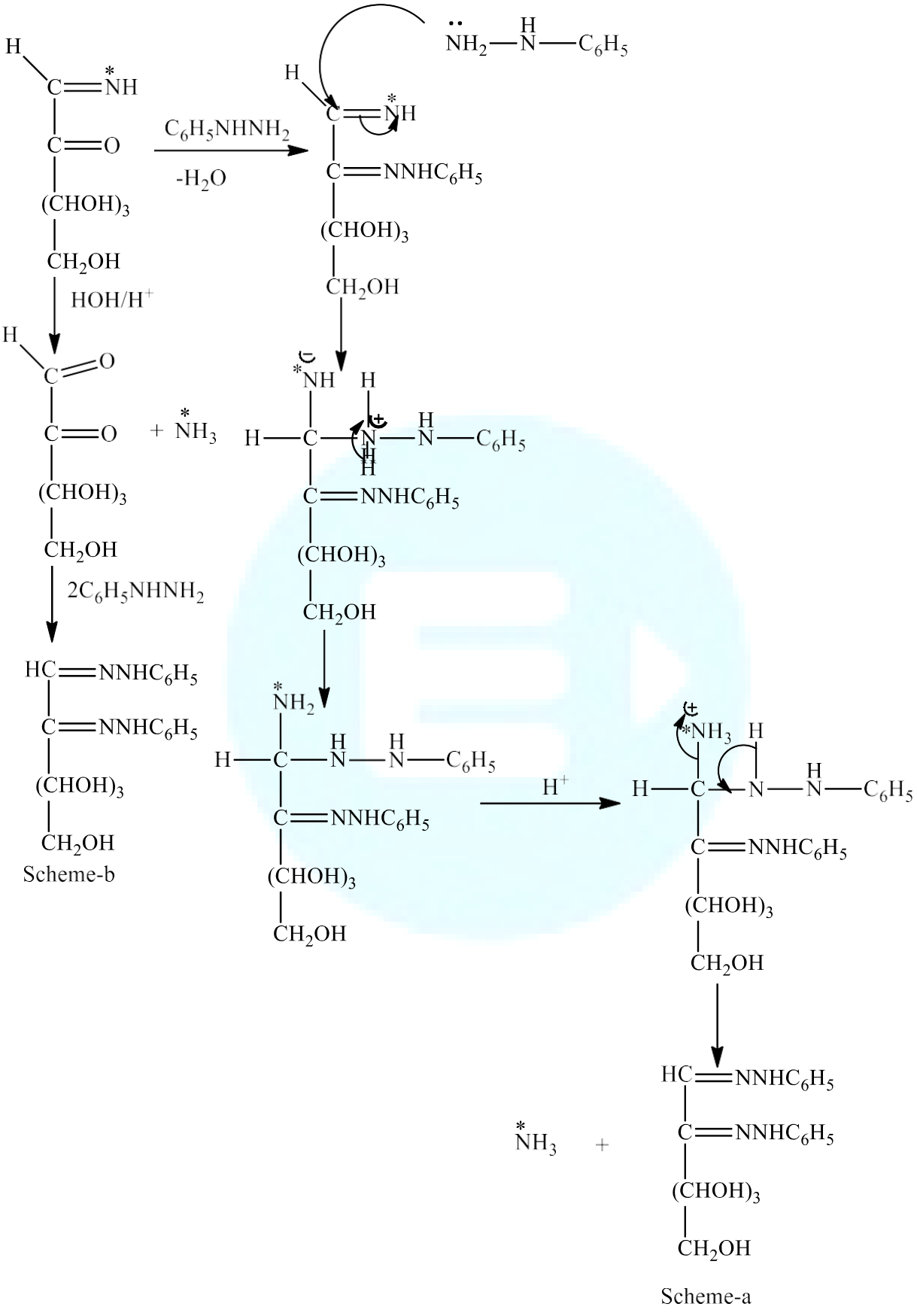
The method involves the oxidation of starting aldose into the corresponding aldonic acid. The acid is converted into its calcium salt which is treated with Fenton's reagent (H2O2 in presence of Fe^{+3} ion) to get the lower aldose. This method is illustrated as follows:



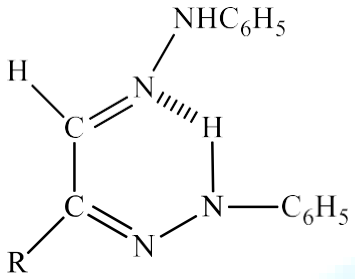
(2) *Wohl's degradation for chain shortening in aldoses*

In this degradation, the aldose is converted into its oxime by treatment with hydrgradazoxylamine. The oxime is treated with acetic anhydride when the oxime is dehydrated to nitrile. The nitrile is then treated with sodium methoxide. The cyanohydrin obtained undergoes degradation to a lower aldose. The reaction are written as under.



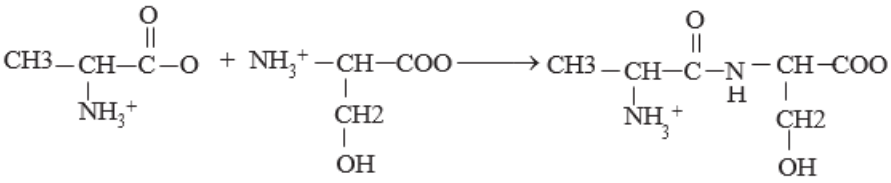

$C_6H_5NHNH_2$
$-H_2O$
HOH/H+
$2C_6H_5NHNH_2$
H^+
Scheme-b
Scheme-a

The osazone so formed does not undergo further amadori rearrangement. This is the reaction with phenylhydrazine stops at this stage; thus further reaction at C-3 –OH group dost not occur. This is because the osazone so formed, does not react further via intramolecular Amadori rearrangement involving C-3 –OH group because of the intramolecular hydrogen bomding as shown below:



Proteins

Proteins are a diverse and abundant class of biomolecules, constituting more than 50% of the dry weight of cells. This diversity and abundance reflect the central role of proteins in virtually all aspects of cell structure and function. Biologically occurring polypeptides range in size from small to very large, consisting of two or three to thousands of linked amino acid residues. Peptidesare chains of amino acids, two amino acid molecules can be covalently joined through a substituted amide linkage, termed a peptide bond (Figure 4.6), to yielda dipeptide. Such a linkage is formed by removal of the elements of water (dehydration) from the □-carboxyl group of one amino acid and the □-amino group of another. Peptide bond formation is an example of a condensation reaction, a common class of reactions in living cells. Three amino acids can bejoined by two peptide bonds to form a tripeptide; similarly, amino acids can belinked to form tetrapeptides, pentapeptides, and so forth. When a few amino acids are joined in this fashion, the structure is called an oligopeptide. When many amino acids are joined, the product is called a polypeptide. Proteins mayhave thousands of amino acid residues. Although the terms “protein” and “polypeptide” are sometimes used interchangeably, molecules referred to as polypeptides generally have molecular weights below 10,000, and those calledproteins have higher molecular weights. Proteins can be assigned to one of threeglobal classes on the basis of shape and solubility: fibrous, globular, or membrane.



Alanine **Serine** **Alanine-Serine**

Peptide bond formation between two amino acids Alanine and Serine.

Fibrous proteins tend to have relatively simple, regular linear structures. These proteins often serve structural roles in cells. Typically, they are insoluble in water or in dilute salt solutions. In contrast, **globular proteins** are roughly spherical in shape. The polypeptide chain is compactly folded so that hydrophobic aminoacid side chains are in the interior of the molecule and the hydrophilic side chainsare on the outside exposed to the solvent, water. **Membrane proteins** are found in association with the various membrane systems of cells. For interaction withthe nonpolar phase within membranes, membrane proteins have hydrophobic amino acid side chains oriented outward. As such, membrane proteins are insoluble in aqueous solutions but can be solubilized in solutions of detergents.

The various levels of protein structural organization are defined as follows.

Primary Structure

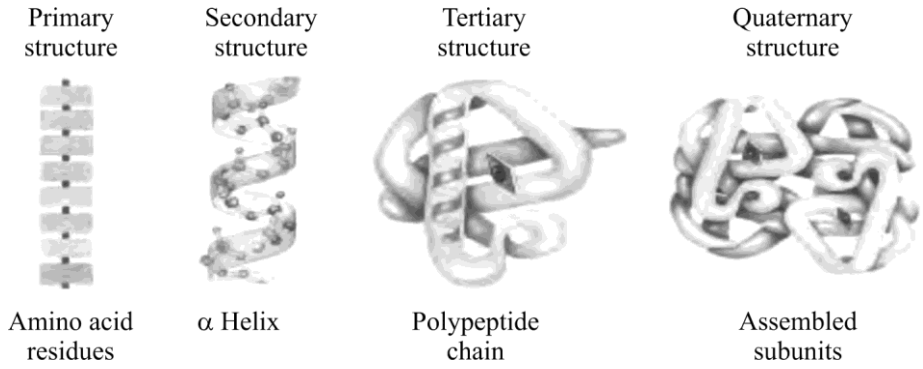
The amino acid sequence is the primary (1°) structure of a protein

Secondary Structure

Through hydrogen bonding interactions between adjacent amino acid residuesthe polypeptide chain can arrange itself into characteristic helical or pleated segments. These segments constitute structural conformities, so-called regular structures that extend along one dimension, like the coils of a spring. Such architectural features of a protein are designated secondary (2°) structures. Secondary structures are just one of the higher levels of structurethat represent the three-dimensional arrangement of the polypeptide in space.

Tertiary Structure

When the polypeptide chains of protein molecules bend and fold in order to assume a more compact three-dimensional shape, a tertiary (3°) level of structure is generated. It is by virtue of their tertiary structure that proteins adopt a globular shape. A globular conformation gives the lowest surface to-volume ratio, minimizing interaction of the protein with the surrounding environment.



Structures of protein.

Quaternary Structure

Many proteins consist of two or more interacting polypeptide chains of characteristic tertiary structure, each of which is commonly referred to as a subunit of the protein. Subunit organization constitutes another level in the hierarchy of protein structure, defined as the protein's quaternary (4°) structure. Whereas the primary structure of a protein is determined by the covalently linked amino acid residues in the polypeptide backbone, secondary and higher orders of structure are determined principally by noncovalent forcessuch as hydrogen bonds and ionic, van der Waals, and hydrophobic interactions.

Functions of proteins

Proteins are the agents of biological function. Virtually every cellular activity is dependent on one or more particular proteins. Thus, a convenient way to classify the enormous number of proteins is by the biological roles they fill. Thevarious functions of proteins are as follows.

Enzymes

By far the largest class of proteins is enzymes. More than 3000 different enzymesare listed in *Enzyme Nomenclature*, the standard reference volume on enzyme classification. **Enzymes** are catalysts that accelerate the rates of biological reactions. Each enzyme is very specific in its function and acts only in a particular metabolic reaction. Virtually every step in metabolism is catalyzed byan enzyme. Enzymes are systematically classified according to the nature of thereaction that they catalyze, such as the transfer of a phosphate group (phosphotransferase) or an oxidation–reduction (oxidoreductase). The formal names of enzymes come from the particular reaction within the class that they catalyze, as in ATP: D-fructose-6-phosphate 1-phosphotransferase. Often, enzymes have common names in addition to their formal names. ATP: D-fructose-6-phosphate 1-phosphotransferase is more commonly known as phosphofructokinase (kinase is a common name given to ATP-dependent phosphotransferases).

Regulatory Proteins

A number of proteins do not perform any obvious chemical transformation butnevertheless can regulate the ability of other proteins to carry out their physiological functions. Such proteins are referred to as regulatory proteins. Awell-known example is insulin, the hormone regulating glucose metabolism in animals. Insulin is a relatively small protein and consists of two polypeptide chains held together by

disulfide cross-bridges. Other hormones that are also proteins include pituitary somatotropin and thyrotropin, which stimulates the thyroid gland.

Transport Proteins

A third class of proteins is the transport proteins. These proteins function to transport specific substances from one place to another. One type of transport is exemplified by the transport of oxygen from the lungs to the tissues by haemoglobin or by the transport of fatty acids from adipose tissue to various organs by the blood protein serum albumin.Membrane transport proteins take up metabolite molecules on one side of a membrane, transport them across themembrane, and release them on the other side. Examples include the transport proteins responsible for the uptake of essential nutrients into the cell, such as glucose or amino acids.

Storage Proteins

Proteins whose biological function is to provide a reservoir of an essential nutrient are called storage proteins. Because proteins are amino acid polymers and because nitrogen is commonly a limiting nutrient for growth, organisms have exploited proteins as a means to provide sufficient nitrogen in times of need. For example, ovalbumin, the protein of egg white, provides the developingbird embryo with a source of nitrogen during its isolation within the egg. Caseinis the most abundant protein of milk and thus the major nitrogen source for mammalian infants. The seeds of higher plants often contain as much as 60% storage protein to make the germinating seed nitrogen-sufficient during this crucial period of plant development. In corn (Zea mays or maize), a family of low molecular weight proteins in the kernel called zeins serve this purpose. Ferritin is a protein found in animal tissues that binds iron, retaining this essential metal so that it is available for the synthesis of important iron-containing proteins such as hemoglobin.

Contractile and Motile Proteins

Certain proteins endow cells with unique capabilities for movement. Celldivision, muscle contraction, and cell motility represent some of the ways in which cells execute motion. Examples include actin and myosin, the filamentousproteins forming the contractile systems of cells, and tubulin, the major component of microtubules.

Structural Proteins

An apparently passive but very important role of proteins is their function in creating and maintaining biological structures. Structural proteins provide strength and protection to cells and tissues. Monomeric units of structural proteins typically polymerize to generate long fibers (as in hair). □-*Keratins* areinsoluble fibrous proteins making up hair, horns, and fingernails. Collagen, another insoluble fibrous protein, is found in bone, connective tissue, tendons,and cartilage, where it forms inelastic fibrils of great strength. One-third of thetotal protein in a vertebrate animal is collagen. A structural protein having elasticproperties is, appropriately, elastin, an important component of ligaments.

Certain insects make a structurally useful protein, fibroin (a a-keratin), the major constituent of cocoons (silk) and spider webs.

Scaffold Proteins (Adapter Proteins)

Some proteins play a recently discovered role in the complex pathways of cellular response to hormones and growth factors. These proteins, the scaffoldor adapter proteins, have a modular organization in which specific parts (modules) of the protein's structure recognize and bind certain structuralelements in other proteins through protein–protein interactions.

Protective and Exploitive Proteins

In contrast to the passive protective nature of some structural proteins, anothergroup can be more aptly classified as protective or exploitive proteins becauseof their biologically active role in cell defense, protection, or exploitation. Prominent among the protective proteins are the immunoglobulins or antibodies produced by the lymphocytes of vertebrates. Antibodies have the remarkable ability to specifically recognize and neutralize "foreign" molecules resulting from the invasion of the organism by bacteria, viruses, or other infectious agents. Another group of protective proteins is the blood-clotting proteins, thrombin andfibrinogen, which prevent the loss of blood when the circulatory system is damaged. Arctic and Antarctic fishes have antifreeze proteins to protect their blood against freezing in the below-zero temperatures of high-latitude seas. Another class of exploitive proteins includes the toxins produced by bacteria, such as diphtheria toxin and cholera toxin. It is worth repeating that the great diversity of function in proteins, as reflected is attained using just 20 amino acids.

Several groups of enzymes catalyze the digestion of proteins. There are two main classes of proteolytic digestive enzymes (proteases), with different specificitiesfor the amino acids forming the peptide bond to be hydrolyzed. Endopeptidases hydrolyze peptide bonds between specific amino acids throughout the molecule.They are the first enzymes to act, yielding a larger number of smaller fragments,eg, pepsin in the gastric juice and trypsin, chymotrypsin, and elastase secretedinto the small intestine by the pancreas. Exopeptidases catalyze the hydrolysisof peptide bonds, one at a time, from the ends of polypeptides. Carboxypeptidases, secreted in the pancreatic juice, release amino acids fromthe free carboxyl terminal, and aminopeptidases, secreted by the intestinal mucosal cells, release amino acids from the amino terminal. Dipeptides, whichare not substrates for exopeptidases, are hydrolyzed in the brush border of intestinal mucosal cells by dipeptidases. The proteases are secreted as inactive zymogens; the active site of the enzyme is masked by a small region of its peptide chain, which is removed by hydrolysis of a specific peptide bond. Pepsinogen is activated to pepsin by gastric acid and by activated pepsin (autocatalysis). In the small intestine, trypsinogen, the precursor of trypsin, is activated by enteropeptidase, which is secreted by the duodenal epithelial cells; trypsin can then activate chymotrypsinogen to chymotrypsin, proelastase to elastase, procarboxypeptidase to carboxypeptidase, and proaminopeptidaseto aminopeptidase.

Free amino acids and small peptides are absorbed by different mechanisms

The end product of the action of endopeptidases and exopeptidases is a mixtureof free amino acids, di- and tripeptides, and oligopeptides, all of which are absorbed. Free amino acids are absorbed across the intestinal mucosa by sodium- dependent active transport. There are several different amino acid transporters,with specificity for the nature of the amino acid side chain (large or small; neutral, acidic, or basic). Dipeptides and tripeptides enter the brush border of the intestinal mucosal cells, where they are hydrolyzed to free amino acids, which are then transported into the hepatic portal vein.

SPECIALIZED PRODUCTS OF AMINO ACIDSPHENYLALANINE, TYROSINE

Phenylalanine

Phenylalanine is first converted to tyrosine. Hyperphenylalaninemias arise from defects in phenylalanine hydroxylase itself (type I, classic phenylketonuriaor PKU), in dihydrobiopterin reductase (types II and III), or in dihydrobiopterinbiosynthesis (types IV and V). DNA probes facilitate prenatal diagnosis of defects in phenylalanine hydroxylase or dihydrobiopterin reductase. A diet low in phenylalanine can prevent the mental retardation of PKU (frequency 1:10,000births). Elevated blood phenylalanine may be detectable by a less reliable screening test that employs $FeCl_3$ to detect urinary phenylpyruvate. $FeCl_3$ screening for PKU of the urine of newborn infants is compulsory in the United

States and many other countries.

Tyrosine
The probable metabolic defect in type I tyrosinemia (tyrosinosis) is at fumarylacetoacetate hydrolase. Therapy employs a diet low in tyrosine and phenylalanine. Untreated acute and chronic tyrosinosis leads to death from liverfailure. Alternate metabolites of tyrosine are also excreted in type II tyrosinemia (Richner-Hanhart syndrome), a defect in tyrosine aminotransferase, and inneonatal tyrosinemia, due to lowered *p*-hydroxyphenylpyruvate hydroxylase activity. Therapy employs a diet low in protein.

TRANSAMINATION (IMPORTANCE OFTRANSAMINASES)

The first step in the catabolism of most L-amino acids, once they have reachedthe liver, is removal of the □-amino groups, promoted by enzymes called **aminotransferases** or **transaminases.** The effect of transamination reactions isto collect the amino groups from many different amino acids in the form of L-glutamate. The glutamate then functions as an amino group donor for biosyntheticpathways or for excretion pathways that lead to the elimination of nitrogenouswaste products. Cells contain different types of aminotransferases. All aminotransferases have the same prosthetic group and the same reaction mechanism. The prosthetic group is **pyridoxal phosphate (PLP),** the coenzymeform of pyridoxine, or vitamin B6. Its primary role in cells is in the metabolism of molecules with amino groups. Pyridoxal phosphate functions as an intermediate carrier of amino groups at the active site of aminotransferases.

DEAMINATION

While ammonia, derived mainly from the □-amino nitrogen of amino acids, is highly toxic, tissues convert ammonia to the amide nitrogen of nontoxicglutamine. Subsequent deamination of glutamine in the liver releases ammonia, which is then converted to nontoxic urea. The deamination of amino acids leaves □-keto acid carbon skeletons. Several of these □-keto acids are citric acid cycle intermediates. Amino acids are used to synthesize liver and plasma proteins, or their carbon skeletons are converted to glucose and glycogen by gluconeogenesis; the ammonia formed by deamination is converted to urea. The □-amino acids collected in the liver in the form of the amino group of L-glutamate moleculesmust be removed from glutamate to prepare them for excretion. In hepatocytes, glutamate is transported from the cytosol into mitochondria, where it undergoes **oxidative deamination** catalyzed by **L-glutamate dehydrogenase**. In mammals,this enzyme is present in the mitochondrial matrix.

Urea cycle

Urea is the major end product of nitrogen catabolism in humans. Urea synthesis is a cyclic process. Synthesis of 1 mol of urea requires 3 mol of ATP plus 1 mol each of ammonium ion and of the □-amino nitrogen of aspartate. Five enzymes catalyze the numbered reactions of Figure 3.8. Of the six participating amino acids, N-acetylglutamate functions solely as an enzyme activator. The others serve as carriers of the atoms that ultimately become urea. The major metabolic role of **ornithine, citrulline**, and **argininosuccinate** in mammals is urea synthesis. Since the ornithine consumed in reaction 2 is regenerated in reaction 5, there is no net loss or gain of ornithine, citrulline, argininosuccinate, or arginine. Ammonium ion, CO_2, ATP, and aspartate are, however, consumed.

Some reactions of urea synthesis occur in the matrix of the mitochondrion, other reactions in the cytosol.

Carbamoyl phosphate synthase I initiates Urea biosynthesis

Condensation of CO_2, ammonia, and ATP to form **carbamoyl phosphate** is catalyzed by mitochondrial **carbamoyl phosphate synthase I**. Carbamoyl phosphate synthase I, the rate-limiting enzyme of the urea cycle, is active only in the presence of its allosteric activator ***N*-acetylglutamate,** which enhances the affinity of the synthase for ATP.

Carbamoyl phosphate plus Ornithine forms Citrulline

L-Ornithine transcarbamoylase catalyzes transfer of the carbamoyl group of carbamoyl phosphate to ornithine, forming citrulline and orthophosphate. While the reaction occurs in the mitochondrial matrix, both the formation of ornithine and the subsequent metabolism of citrulline take place in the cytosol.

Citrulline plus Aspartate forms Argininosuccinate

Argininosuccinate synthase links aspartate and citrulline via the amino group of aspartate and provides the second nitrogen of urea. The reaction requires ATP and involves intermediate formation of citrullyl-AMP. Subsequent displacement of AMP by aspartate then forms citrulline.

Cleavage of Argininosuccinate forms Arginine and Fumarate

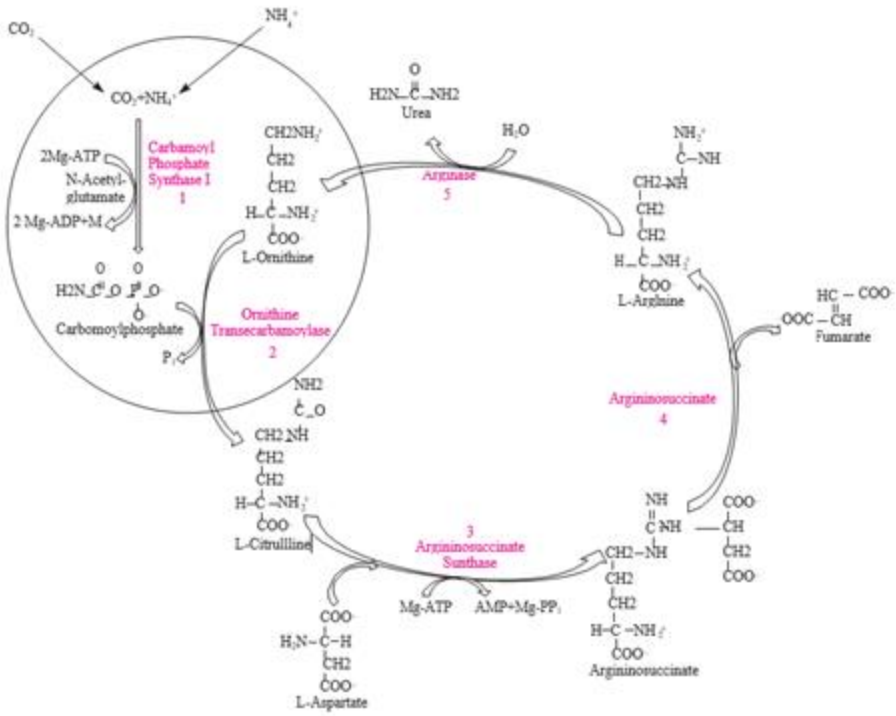
Cleavage of argininosuccinate, catalyzed by **argininosuccinase**, proceeds with retention of nitrogen in arginine and release of the aspartate skeleton as fumarate.

Cleavage of Arginine releases Urea and re-forms Ornithine

Hydrolytic cleavage of the guanidino group of arginine, catalyzed by liver arginase, releases urea. The other product, ornithine, reenters liver mitochondria for additional rounds of urea synthesis.

Carbamoyl phosphate synthase I is the pacemaker enzyme of the Urea cycle

The activity of carbamoyl phosphate synthase I is determined by N-acetylglutamate, whose steady-state level is dictated by its rate of synthesis from acetyl-CoA and glutamate and its rate of hydrolysis to acetate and glutamate. These reactions are catalyzed by N-acetylglutamate synthase and N-acetylglutamate hydrolase, respectively. Major changes in diet can increase the concentrations of individual urea cycle enzymes 10-fold to 20-fold. Starvation, for example, elevates enzyme levels, presumably to cope with the increased production of ammonia that accompanies enhanced protein degradation.



Reactions and intermediates of urea biosynthesis. Reactions 1 and 2 occurin the matrix of liver mitochondria and reactions 3, 4, and 5 in liver cytosol. CO_2 (as bicarbonate), ammonium ion, ornithine, and citrulline enter the mitochondrial matrix via specific carriers present in the inner membrane of liver mitochondria.

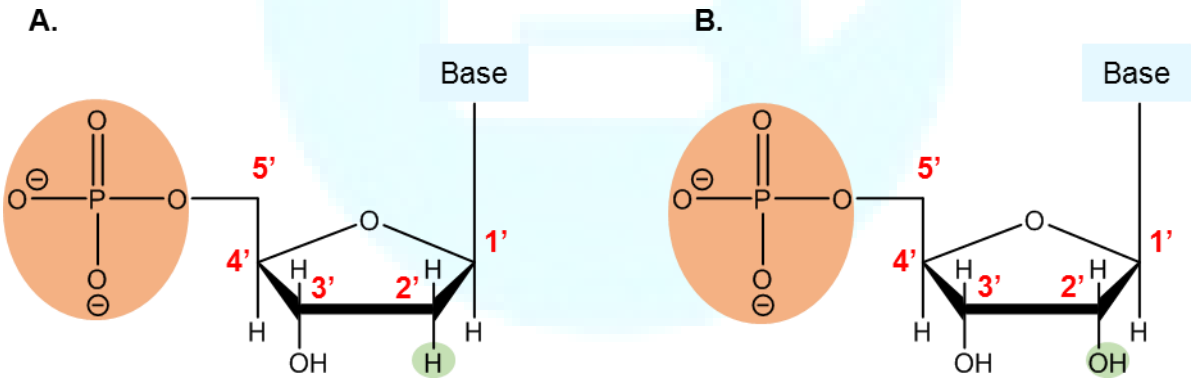
NUCLEIC ACIDS

Nucleic acids are the carriers of genetic information. In all living organisms, the hereditary information is stored in deoxyribonucleic acid (DNA), which is a molecule formed by the repetition of *nucleotides* (making DNA a *polymer*). There are four different nucleotides in DNA, which form a universal code for hereditary information.

Ribonucleic acid (RNA), the other kind of nucleic acid, is a related molecule to DNA. It is also a polymer of four nucleotides, three of which are the same as in DNA while the fourth one is slightly different. It has many functions in cells, notably acting as the intermediate between DNA and proteins. Some viruses even store their genome in the form of an RNA molecule rather than DNA.

Nucleotides

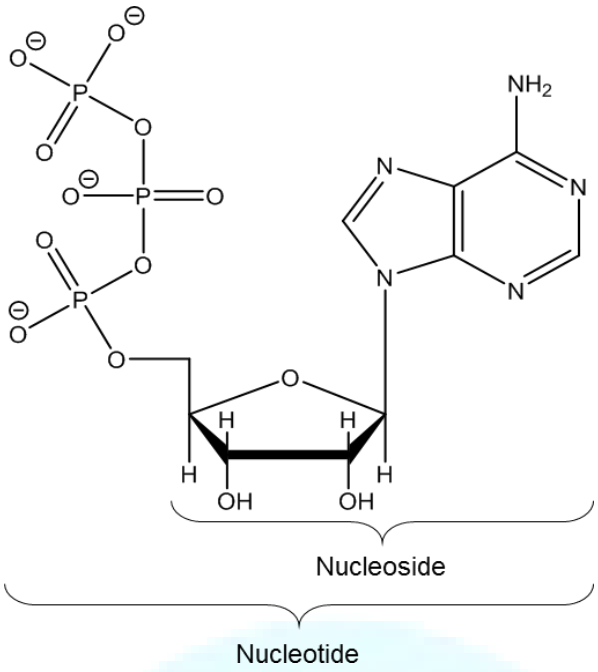
Nucleotides are the building blocks of nucleic acids: they are the *monomers* which, repeated many times, form the *polymers* DNA and RNA. Nucleotides are composed of a five-carbon sugar covalently attached to a phosphate group and a base containing nitrogen atoms.



The chemical structure of a nucleotides. A nucleotide comprises a five-carbon sugar molecule: deoxyribose in DNA (**A**) and ribose in RNA (**B**). The carbon atoms on the sugar moleculeare numbered in red. Deoxyribose (**A**) is different from ribose (**B**) in that it lacks an –OH group at carbon 2'. The 5'-carbon atom is attached to a phosphate group (here a monophosphate in orange) and the 1'-carbon is attached to a base (blue).

The main difference between nucleotides from DNA and those from RNA is the nature of the sugar. Nucleotides making up RNA (Figure 1B) contain ribose, making them *ribonucleotides*. In DNA, however, the sugar lacks an –OH group at the 2'-carbon, making it *deoxyribose* and the corresponding nucleotides *deoxyribonuleotides*.

A nucleotide may contain more than one phosphate at its 5’-carbon, for instance the nucleotide adenosine triphosphate has three. When there is no phosphate group, the moleculeis no longer called a *nucleotide*, but a *nucleoside*.

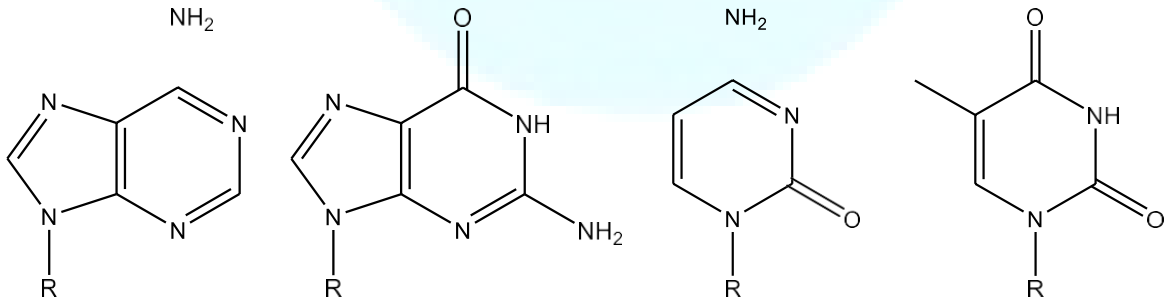


Adenosine triphosphate, often abbreviated to ATP.

Bases in nucleic acids

The nucleotides making up DNA contain one of four *nitrogenous bases* (i.e. bases that contain nitrogen atoms). From a chemical perspective, two of those bases are *purines*, while the other two are *pyrimidines*. To each base corresponds a name (e.g. adenine), a nucleoside (e.g. adenosine) and a one-letter code (e.g. A). This information is included in Table 1.

The four bases of DNA. The 'R' represents the deoxyribose covalently attached to thebase to form the nucleoside named in the third row.

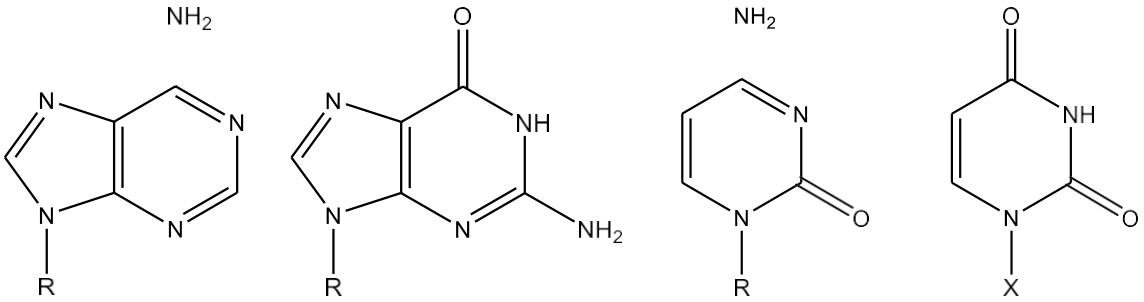


Adenine	Guanine	Cytosine	Thymine
Deoxyadenosine	Deoxyguanosine	Deoxycytidine	Deoxythymidine
A	G	C	T
Purines		Pyrimidines	

The sugar in RNA is ribose rather than deoxyribose. However, there is anotherdifference between DNA and RNA in the base composition. RNA contains three of the bases found in DNA (adenine, guanine and cytosine) but thymine is replaced by the related base, uracil. The fourbases found in RNA,

along with the names of their corresponding nucleosides.

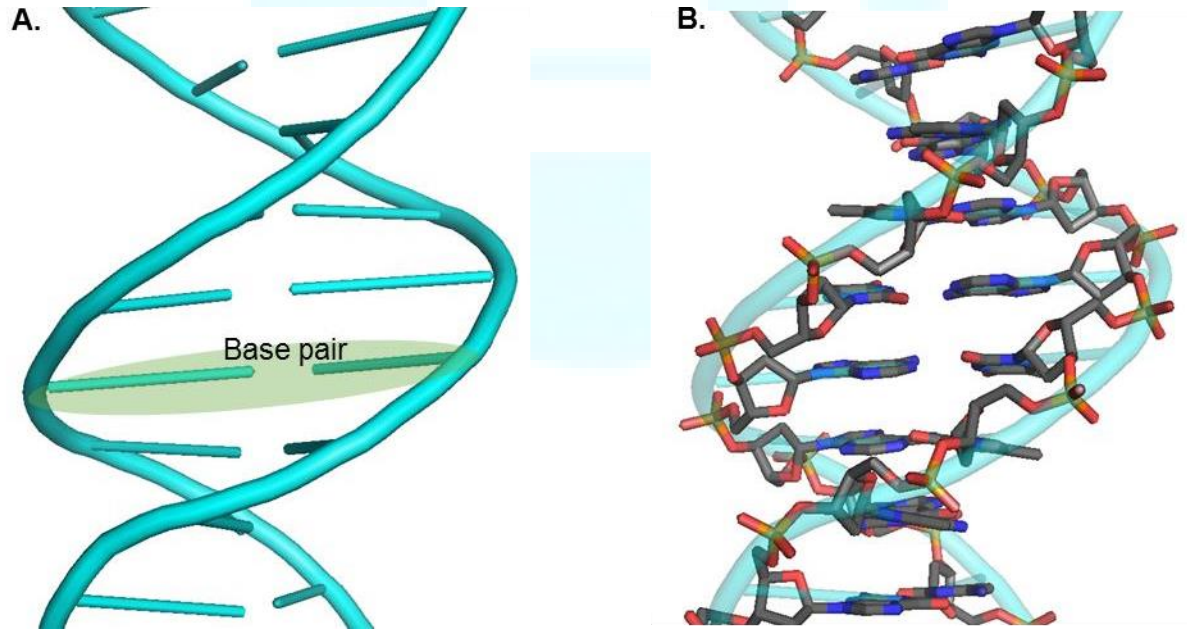
The four bases of RNA. The 'R' represents the ribose covalently attached to the base toform the nucleoside named in the third row.



Adenine	Guanine	Cytosine	Uracil
Adenosine	Guanosine	Cytidine	Uridine
A	G	C	U
Purines		Pyrimidines	

3D structure of DNA

DNA is predominantly found as a double helix: two strands of polynucleotides wind about the same axis to form a right-handed helix. Each nucleotide provides a ribose and a phosphate to the backbone. The bases project towards the centre of the helix, away from the surrounding water.



The double-helical structure of DNA. **A.** DNA shown as a cartoon. **B.** DNA shown as sticks, with a cyan cartoon highlighting the sugar-phosphate backbone. Green: base pair; grey: carbon; red: oxygen; blue: nitrogen; white: hydrogen; orange: sulphur.

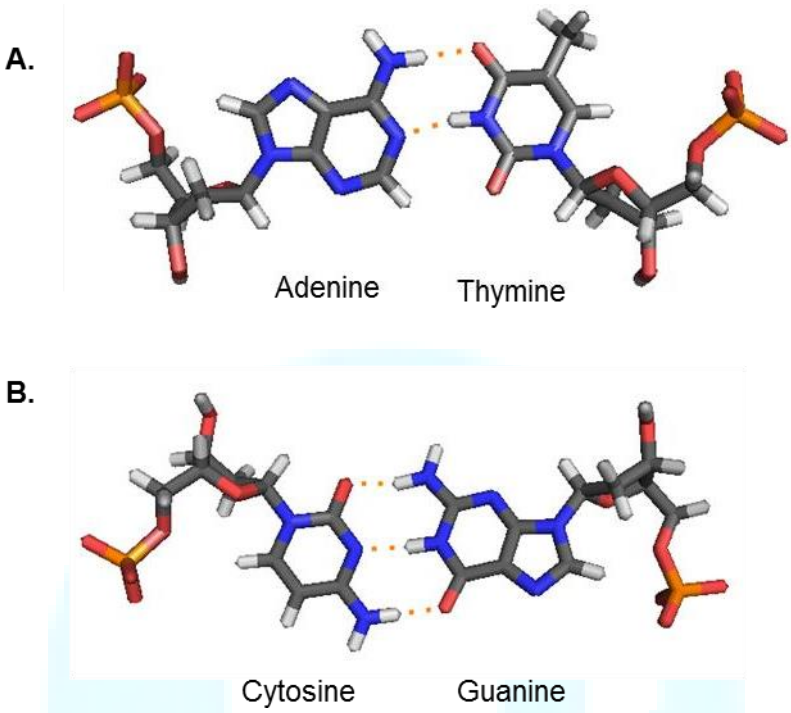
Two bases (each from a different strand) come together to form a *base pair*, shown in green in Figure 3A. A base pair is held together by hydrogen bonds between the two bases (cf. Watson-Crick base

pairing explained below).

DNA can adopt slightly different kinds of 3D structure, but the majority of the DNA inside a cell at any given point will have the structure shown, called *B-DNA*. It has 10 base pairs per helical turn and a rise of 3.4Å per base pair.

Watson-Crick base pairing

The double helix shown in Figure 3 can only accommodate two kinds of base pairs, due to the geometry of the bases. Adenine and thymine bases always pair with each other while guanine and cytosine bases always pair with each other. This kind of pairing, called *Watson-Crick base pairing*, is mediated by hydrogen bonds between the two bases of a pair.



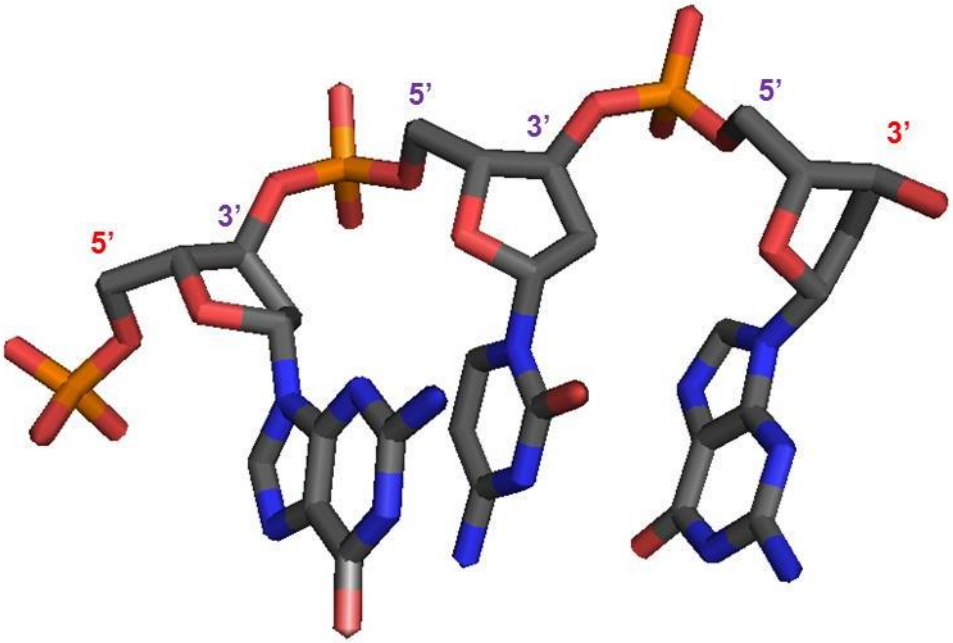
A. Watson-Crick base pairing between deoxyriboadenosine monophosphate and deoxyribothymidine monophosphate. **B.** Watson-Crick base pairing between deoxyribocytidine mono- phosphate and deoxyriboguanosine monophosphate. Only the name of the base is given below each nucleotide. The hydrogen bonds are shown by orange dotted lines. Grey: carbon; red: oxygen;blue: nitrogen; white: hydrogen; orange: sulphur.

Note that an AT base pair is only held by two hydrogen bonds whereas a CG base-pair has three, making the latter more stable.

Directionality of DNA

A strand of DNA is the result of the polymerisation of several nucleotides, with the backbone formed by the deoxyribose sugars and the phosphate groups. Each nucleotide *residue* (i.e. a nucleotide withina strand of DNA) contains a phosphate group covalently attached to the 5'-carbon of its deoxyribose,but also has its deoxyribose 3'-carbon covalently attached to the phosphate of the next nucleotide residue in the strand. The only exception is the final nucleotide, which does not have a phosphate atits 3'-carbon (of the deoxyribose), but rather a free –OH group. We define this end of the strand as the 3'-end. The very first nucleotide residue, on the other hand, has a free phosphate group attachedto its 5'-carbon. We define that end of the strand as the 5'-end.

DNA is always read from the 5'-end to the 3'-end.



Sequence: **5'-G-C-G-3'**

The directionality of DNA. A stretch of 3 nucleotide residues is shown with their 5'- and 3'-carbons numbered. In red are the 5'-end (characterised by a free phosphate group) and the 3'- end (characterised by a free –OH group).

Note that, when studying DNA in the lab, it is common to remove the phosphate at the 5'-end, there-fore many experimentally determined structures will actually show an –OH group rather than a phos-phate at the 5'-end.

Structure of RNA differ from that of DNA

As mentioned above, RNA is made of ribonuleotides rather than deoxyribonucleotides: the 2'-carbonof its ribose is covalently attached to an –OH group. Furthermore, RNA contains the base uracil in- stead of thymine.

The other main difference between RNA and DNA is that RNA is often single-stranded and does notform the regular double-helical structure of DNA. However, it is quite common for a single RNA strandto fold on itself and to form complex 3D structures, with some helical character. When that is the case,the 3D structure is often stabilised by the same Watson-Crick base-pairing as in DNA, although somedeviations may be allowed (often disrupting helices).

The directionality of RNA, however, is the same as that of DNA: the sequence is read from the 5'-endto the 3'-end.

Vitamins

The term “vitamin” is used to describe certain organic compounds that are needed by the body but that cannot be manufactured by the body. They mainlyserve as catalysts for certain reactions in the body. The amounts of vitamins required are very small, perhaps hundredths of grams. Vitamins are mainly obtained from our foods.

Classification of vitamins

Based on solubility Vitamins are classified as either fat-soluble (lipid soluble) or water-soluble. Vitamins A, D, E and K are fat-soluble Vitamin C and B is water soluble.

WATER-SOLUBLE VITAMINS

B-complex vitamins
Eight of the water-soluble vitamins are known as the vitamin B-complex group:thiamin (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), vitamin B6(pyridoxine), folate (folic acid), vitamin B12, biotin and pantothenic acid. The B vitamins are widely distributed in foods and their influence is felt in many parts of the body. They function as coenzymes that help the body obtain energy fromfood. The B vitamins are also important for normal appetite, good vision, and healthy skin, nervous system, and red blood cell formation.

Thiamin: Vitamin B1
Thiamin, or vitamin B1, helps to release energy from foods, promotes normal appetite, and is important in maintaining proper nervous system function. Sources include peas, pork, liver, and legumes. Most commonly, thiamin is found in whole grains and fortified grain products such as cereal, and enrichedproducts like bread, pasta, rice, and tortillas. The process of enrichment adds back nutrients that are lost when grains are processed. Among the nutrients added during the enrichment process are thiamin (B1), niacin (B3), riboflavin (B2), folate and iron.

RDA (Required Daily allowance)

Males: 1.2 mg/day; **Females**: 1.1 mg/day

Riboflavin: Vitamin B2

Riboflavin, or vitamin B2, helps to release energy from foods, promotes good vision, and healthy skin. It also helps to convert the amino acid tryptophan (which makes up protein) into niacin. Sources include liver, eggs, dark green vegetables, legumes, whole and enrichedgrain products, and milk. Ultraviolet light is known to destroy riboflavin, whichis why most milk is packaged in opaque containers instead of clear.

RDA

Males: 1.3 mg/day; **Females:** 1.1 mg/day

Niacin: Vitamin B3, Nicotinamide, Nicotinic Acid

Niacin, or vitamin B3, is involved in energy production, normal enzymefunction, digestion, promoting normal appetite, healthy skin, and nerves.Sources include liver, fish, poultry, meat, peanuts, whole and enriched grain products.

RDA

Males: 16 mg/day; **Females:** 14 mg/day

Vitamin B6: Pyridoxine, Pyridoxal, Pyridoxamine

Vitamin B6, otherwise known as pyridoxine, pyridoxal or pyridoxamine, aids in protein metabolism and red blood cell formation. It is also involved in the body's production of chemicals such as insulin and hemoglobin. Sources include pork, meats, whole grains and cereals, legumes, and green, leafyvegetables. The RDA for vitamin B6 is 1.3 mg/day for adult males and femalesthrough age fifty.

Folate: Folic Acid, Folacin

Folate, also known as folic acid or folacin, aids in protein metabolism, promoting red blood cell formation, and lowering the risk for neural tube birth defects. Folate may also play a role in controlling homocysteine levels, thus reducing therisk for coronary heart disease. Sources of folate include liver, kidney, dark green leafy vegetables, meats, fish,whole grains, fortified grains and cereals, legumes, and citrus fruits. Not all whole grain products are fortified with folate..

The RDA for folate is 400 mcg/day for adult males and females. Pregnancy willincrease the RDA for folate to 600 mcg/day.

Vitamin B12: Cobalamin

Vitamin B12, also known as cobalamin, aids in the building of genetic material,production of normal red blood cells, and maintenance of the nervous system. Vitamin B12 can only be found only in foods of animal origin such as meats, liver, kidney, fish, eggs, milk and milk products, oysters, shellfish. Some fortified foods may contain vitamin B12. The Recommended Dietary Allowance (RDA) for vitamin B12 is 2.4 mcg/dayfor adult males and females

Biotin

Biotin helps release energy from carbohydrates and aids in the metabolism of fats, proteins and carbohydrates from food. Sources of Biotin include liver, kidney, egg yolk, milk, most fresh vegetables, yeast breads and cereals. Biotin is also made by intestinal bacteria. The Adequate Intake (AI) for Biotin is 30 mcg/day for adult males and females

Pantothenic Acid

Pantothenic Acid is involved in energy production, and aids in the formation ofhormones and the metabolism of fats, proteins, and carbohydrates from food. Sources include liver, kidney, meats, egg yolk, whole grains, and legumes. Pantothenic Acid is also made by intestinal bacteria. The Adequate Intake (AI) for Pantothenic Acid is 5 mg/day for both adult malesand females.

Vitamin C

The body needs vitamin C, also known as ascorbic acid or ascorbate. Vitamin C benefits the body by holding cells together through collagen synthesis; collagen is a connective tissue that holds muscles, bones, and other tissues together. Vitamin C also aids in wound healing, bone and tooth formation, strengthening blood vessel walls, improving immune system function, increasingabsorption and utilization of iron, and acting as an antioxidant. Since our bodies cannot produce or store vitamin C, an adequate daily intake of this nutrient is essential for optimum health. Vitamin C works with vitamin E as an antioxidant, and plays a crucial role in neutralizing free radicalsthroughout the body. An antioxidant can be a vitamin, mineral, or a carotenoid,present in foods, that slows the oxidation process and acts to repair damage to cells of the body. Studies suggest that vitamin C may reduce the risk of certain cancers, heart disease, and cataracts. Research continues to document the degreeof these effects. Consuming vitamin C-rich foods is the best method to ensure an adequate intakeof this vitamin. While many common plant foods contain vitamin C, the best sources are citrus fruits (orange, kiwi fruit, grape etc,) The Recommended Dietary Allowance (RDA) for Vitamin C is 90 mg/day foradult males and 75 mg/day for adult females For those who smoke cigarettes, the RDA for vitamin C increases by 35 mg/day, in order to counteract the oxidative effects of nicotine. Severe vitamin C deficiency result in the disease known as scurvy, causing a lossof collagen strength throughout the body. Loss of collagen results in loose teeth, bleeding and swollen gums, and improper wound healing. More commonly, vitamin C deficiency presents as a secondary deficiency in alcoholics, the elderly, and in smokers.

FAT-SOLUBLE VITAMINS

The fat-soluble vitamins, A, D, E, and K, are stored in the body for long periodsof time and generally pose a greater risk for toxicity when consumed in excessthan water-soluble vitamins. Eating a normal, well-balanced diet will not lead to toxicity in otherwise healthy individuals. However, taking vitamin supplementsthat contain megadoses of vitamins A, D, E and K may lead to toxicity. The bodyonly needs small amounts of any vitamin.

While diseases caused by a lack of fat soluble vitamins are rare symptoms of mild deficiency can develop without adequate amounts of vitamins in the diet. Additionally, some health problems may decrease the absorption of fat, and inturn, decrease the absorption of vitamins A, D, E and K. Consult a medical professional about any potential health problems that may interfere with vitaminabsorption.

Vitamin A

Vitamin A, also called retinol, has many functions in the body. In addition to helping the eyes adjust to light changes, vitamin A plays an important role in bone growth, tooth development, reproduction, cell division, gene expression, and regulation of the immune

system. The skin, eyes, and mucous membranesof the mouth, nose, throat and lungs depend on vitamin A to remain moist. Vitamin A is also an important antioxidant that may play a role in the preventionof certain cancers. Eating a wide variety of foods is the best way to ensure that the body gets enoughvitamin A. The retinol, retinal, and retinoic acid forms of vitamin A are suppliedprimarily by foods of animal origin such as dairy products, fish and liver. Somefoods of plant origin contain the antioxidant, betacarotene, which the body converts to vitamin A. Beta-carotene, comes from fruits and vegetables, especially those that are orange or dark green in color. Vitamin A sources alsoinclude carrots, pumpkin, winter squash, dark green leafy vegetables andapricots, all of which are rich in beta-carotene.

Vitamin D

Vitamin D plays a critical role in the body's use of calcium and phosphorous. It works by increasing the amount of calcium absorbed from the small intestine, helping to form and maintain bones. Vitamin D benefits the body by playing arole in immunity and controlling cell growth. Children especially need adequateamounts of vitamin D to develop strong bones and healthy teeth.The primary food sources of vitamin D are milk and other dairy products fortifiedwith vitamin D. Vitamin D is also found in oily fish (e.g., herring, salmon and sardines) as well as in cod liver oil. In addition to the vitamin D provided by food, we obtain vitamin D through our skin which produces vitamin D in response to sunlight. The Recommended Dietary Allowance (RDA) for vitamin D appears as micrograms (mcg) of cholecalciferol (vitamin D3). From 12 months to age fifty,the RDA is set at 15 mcg. Twenty mcg of cholecalciferol equals 800 InternationalUnits (IU), which is the recommendation for maintenance of healthy bone for adults over fifty. Exposure to ultraviolet light is necessary for the body to produce the active formof vitamin D. Ten to fifteen minutes of sunlight without sunscreen on the hands,arms and face, twice a week is sufficient to receive enough vitamin D. This caneasily be obtained in the time spent riding a bike to work or taking a short walk. In order to reduce the risk for skin cancer one should apply sunscreen with an SPF of 15 or more, if time in the sun exceeds 10 to 15 minutes.

Vitamin E

Vitamin E benefits the body by acting as an antioxidant, and protecting vitaminsA and C, red blood cells, and essential fatty acids from destruction. Research from decades ago suggested that taking antioxidant supplements, vitamin E in particular, might help prevent heart disease and cancer. However, newer findingsindicate that people who take antioxidant and vitamin E supplements are not better protected against heart disease and cancer than non-supplement users. Many studies show a link between regularly eating an antioxidant rich diet fullof fruits and vegetables, and a lower risk for heart disease, cancer, and several other diseases. Essentially, recent research indicates that to receive the full benefits of antioxidants and phytonutrients in the diet, one should consume thesecompounds in the form of fruits and vegetables, not as supplements. About 60 percent of vitamin E in the diet comes from vegetable oil (soybean, corn, cottonseed, and safflower). This also includes products made with vegetable oil (margarine and salad dressing). Vitamin E sources also include fruits and vegetables, grains, nuts (almonds and hazelnuts), seeds (sunflower) and fortified cereals. The Recommended Dietary Allowance (RDA) for vitamin E is based on the mostactive and

usable form called alpha-tocopherol. Food and supplement labels listalpha-tocopherol as the unit international units (IU) not in milligrams (mg). Onemilligram of alpha-tocopherol equals to 1.5 International Units (IU). RDA guidelines state that males and females over the age of 14 should receive 15 mcgof alpha-tocopherol per day. Consuming vitamin E in excess of the RDA does not result in any added benefits.

Vitamin K

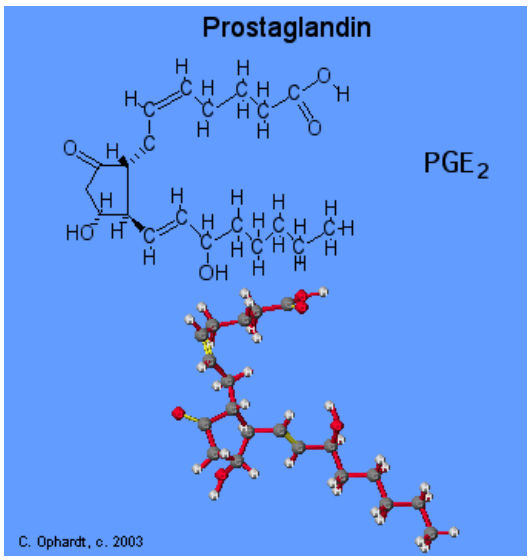
Vitamin K is naturally produced by the bacteria in the intestines, and plays an essential role in normal blood clotting, promoting bone health, and helping to produce proteins for blood, bones, and kidneys. Good food sources of vitamin K are green, leafy-vegetables such as turnip greens, spinach, cauliflower, cabbage and broccoli, and certain vegetables oils including soybean oil, cottonseed oil, canola oil and olive oil. Animal foods, ingeneral, contain limited amounts of vitamin K. Males and females age 14 - 18: 75 mcg/day; Males and females age 19 and older:90 mcg/day

Prostaglandins

Prostaglandins were first discovered and isolated from human semen in the 1930s by Ulf von Euler of Sweden. Thinking they had come from the prostate gland, he named them prostaglandins. It has since been determined that they exist and are synthesized in virtually every cell of the body. Prostaglandins, are like hormones in that they act as chemical messengers, but do not move to other sites, but work right within the cells where they are synthesized.

Introduction

Prostaglandins are unsaturated carboxylic acids, consisting of of a 20 carbon skeleton that also contains a five member ring. They are biochemically synthesized from the fatty acid, arachidonic acid. See the graphic on the left. The unique shape of the arachidonic acid caused by a series of cis double bonds helps to put it into position to make the five member ring. See the prostaglandin in the next panel



Prostaglandin Structure

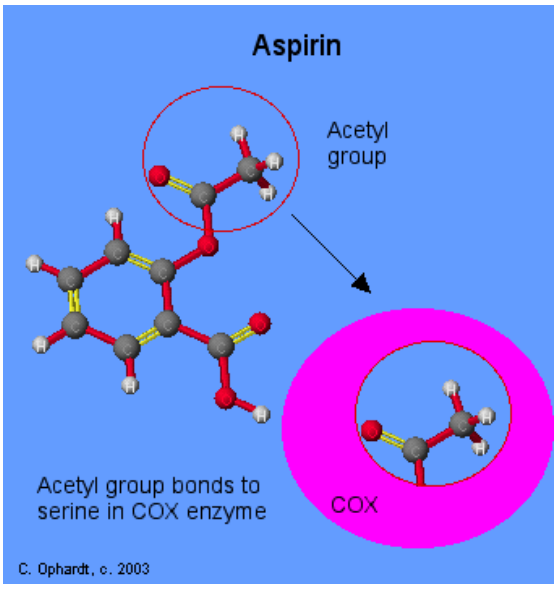
Prostaglandins are unsaturated carboxylic acids, consisting of of a 20 carbon skeleton that also contains a five member ring and are based upon the fatty acid, arachidonic acid. There are a variety of structures one, two, or three double bonds. On the five member ring there may also be double bonds, a ketone, or alcohol groups. A typical structure is on the left graphic.

Functions of Prostaglandins

There are a variety of physiological effects including:

1. Activation of the inflammatory response, production of pain, and fever. When tissues are damaged, white blood cells flood to the site to try to minimize tissue destruction. Prostaglandins are produced as a result.

2. Blood clots form when a blood vessel is damaged. A type of prostaglandin called thromboxane stimulates constriction and clotting of platelets. Conversely, PGI2, is produced to have the opposite effect on the walls of blood vessels where clots should not be forming.
3. Certain prostaglandins are involved with the induction of labor and other reproductive processes. PGE2 causes uterine contractions and has been used to induce labor.
4. Prostaglandins are involved in several other organs such as the gastrointestinal tract (inhibit acid synthesis and increase secretion of protective mucus), increase blood flow in kidneys, and leukotriens promote constriction of bronchi associated with asthma.



Effects of Aspirin and other Pain Killers

When you see that prostaglandins induce inflammation, pain, and fever, what comes to mind but aspirin. Aspirin blocks an enzyme called cyclooxygenase, COX-1 and COX-2, which is involved with the ring closure and addition of oxygen to arachidonic acid converting to prostaglandins. The acetyl group on aspirin is hydrolzed and then bonded to the alcohol group of serine as an ester. This has the effect of blocking the channel in the enzyme and arachidonic can not enter the active site of the enzyme. By inhibiting or blocking this enzyme, the synthesis of prostaglandins is blocked, which in turn relives some of the effects of pain and fever. Aspirin is also thought to inhibit the prostaglandin synthesis involved with unwanted blood clotting in coronary heart disease. At the same time an injury while taking aspirin may cause more extensive bleeding.

Hormones

A hormone is a chemical that acts as a messenger transmitting a signal from onecell to another. When it binds to another cell which is the target of the message,the hormone can alter several aspects of cell function, including cell growth, metabolism, or other function. Hormones can be classified according to chemical composition, solubility properties, location of receptors, and the natureof the signal used to mediate hormonal action within the cell. Hormones that bind to the surfaces of cells communicate with intracellular metabolic processes through intermediary molecules called second messengers (the hormone itself is the first messenger), which are generated as a consequence of the ligand- receptor interaction. The second messenger concept arose from an observation that epinephrine binds to the plasma membrane of certain cells and increases intracellular cAMP. This was followed by a series of experiments in which cAMP was found to mediate the effects of many hormones. To date, only one hormone, atrial natriuretic factor (ANF), uses cGMP as its second messenger.

Characterizing hormone

The first way of characterizing a hormone is by looking at the distance over which the hormone acts. Hormones can be classified on three primary ways asfollowing:

- Autocrine: An autocrine hormone is one that acts on the same cell that released it.
- Paracrine: A paracrine hormone is one that acts on cells which are nearby relative to the cell which released it. An example of paracrine hormones includes growth factors, which are proteins that stimulate cellular proliferation and differentiation. Specifically, consider the binding of white blood cells to T cells. When the white blood cell binds to a T cell, it releases a protein growth factor called interleukin-1. This causes the T cell to proliferate and differentiate.
- Endocrine: An endocrine hormone is one that is released into the bloodstream by endocrine glands. The receptor cells are distant from the source. An example of an endocrine hormone is insulin, which is released by the pancreas into the bloodstream where it regulates glucose uptake by liver and muscle cells.

There are three major classifications one should be aware of:

- Steroids: Steroid hormones are for the most part derivatives of cholesterol.
- Amino acid derivatives: Several hormones (and neurotransmitters) arederived from amino acids.
- Polypeptides: Many hormones are chains of amino acids.

Insulin

Insulin is a polypeptide hormone synthesized in the pancreas by □-cells, whichconstruct a single chain molecule called proinsulin. Enzymes excise a portion of the proinsulin molecule called the C peptide, producing the actual insulin molecule. When in demand, the □-cells will release insulin together with the c peptide into the blood stream via exocytosis. The role of insulin in the body is well known, with its primary role being to control the uptake of glucose by liverand muscle cells and also the storage of glucose in the form of glycogen.

Diabetes results from a lack of insulin secretion by the pancreas. Without insulin,cells take up glucose very slowly. The lack of insulin results in an inability to use blood glucose for fuel. Insulin is a signal for high blood glucose levels andincreases glucose transport into cells. It stimulates synthesis of glycogen, fat, and protein. It inhibits breakdown of glycogen, fat, and protein. Insulin, secreted bythe □-cells of the pancreas in response to rising blood glucose levels, is a signalthat glucose is abundant. Insulin binds to a specific receptor on the cell surface and exerts its metabolic effect by a signaling pathway that involves a receptor tyrosine kinase phosphorylation cascade. Note that insulin stimulates storage processes and at the same time inhibits degradative pathways.

The pancreas secretes insulin or glucagon in response to changes in blood glucose

When glucose enters the bloodstream from the intestine after a carbohydrate- rich meal, the resulting increase in blood glucose causes increased secretion of insulin (and decreased secretion of glucagon). Insulin release by the pancreas is largely regulated by the level of glucose in the blood supplied to the pancreas.The peptide hormones insulin, glucagon, and somatostatin are produced by clusters of specialized pancreatic cells, the islets of Langerhans. Each cell typeof the islets produces a single hormone: α-cells produce glucagon; □-cells, insulin; and δ-cells, somatostatin.

Insulin secretion

When blood glucose rises, GLUT2 transporters carry glucose into the b-cells, where it is immediately converted to glucose 6-phosphate by hexokinase IV (glucokinase) and enters glycolysis. The increased rate of glucose catabolism raises [ATP], causing the closing of ATP-gated K^+ channels in the plasma membrane. Reduced efflux of K^+ depolarizes the membrane, thereby opening voltage-sensitive Ca^{2+} channels in the plasma membrane. The resulting influx of Ca^{2+} triggers the release of insulin by exocytosis. Stimuli from the parasympathetic and sympathetic nervous systems also stimulate and inhibit insulin release, respectively. Insulin lowers blood glucose by stimulating glucose uptake by the tissues; the reduced blood glucose is detected by the □-cell as a diminished flux through the hexokinase reaction; this slows or stops the release of insulin. This feedback regulation holds blood glucose concentration nearly constant despite large fluctuations in dietary intake.

Insulin counters high blood glucose

Insulin stimulates glucose uptake by muscle and adipose tissue, where the glucose is converted to glucose 6-phosphate. In the liver, insulin also activatesglycogen synthase and inactivates glycogen phosphorylase, so that much of theglucose 6-phosphate is channelled into glycogen. Insulin also stimulates the storage of excess fuel as fat. In the liver, insulin activates both the oxidation ofglucose 6-phosphate to pyruvate via glycolysis and the oxidation of pyruvate to acetyl-CoA. If not oxidized further for energy production, this acetyl- CoA is used for fatty acid synthesis in the liver, and the fatty acids are exported as thetriglyerides to the adipose tissue. These fatty acids are ultimately derived fromthe excess glucose taken up from the blood by the liver. In summary, the effectof insulin is to favor the conversion of excess blood glucose to two storage forms: glycogen (in the liver and muscle) and triacylglycerols (in adipose tissue).

Glucagon

Glucagon, a peptide hormone synthesized and secreted from the □-cells of the islets of Langerhans of pancreas, raises blood glucose levels. Its effect is opposite that of insulin, which lowers blood glucose levels. The pancreas releases glucagon when blood sugar (glucose) levels fall too low. Glucagon causes the liver to convert stored glycogen into glucose, which is released intothe bloodstream. High blood glucose levels stimulate the release of insulin. Insulin allows glucose to be taken up and used by insulin-dependent tissues. Thus, glucagon and insulin are part of a feedback system that keeps blood glucose levels at a stable level.

Regulation and function

Secretion of glucagon is stimulated by hypoglycemia, epinephrine, arginine, alanine, acetylcholine, and cholecystokinin. Secretion of glucagon is inhibited by somatostatin, insulin, increased free fatty acids and keto acids into the blood,and increased urea production. Glucose is stored in the liver in the form of glycogen, which is a starch-like polymer chain made up of glucose molecules.Liver cells (hepatocytes) have glucagon receptors. When glucagon binds to theglucagon receptors, the liver cells convert the glycogen polymer into individual glucose molecules, and release them into the bloodstream, in a process known as glycogenolysis. As these stores become depleted, glucagon then encouragesthe liver and kidney to synthesize additional glucose by gluconeogenesis.Glucagon turns off glycolysis in the liver, causing glycolytic intermediates to beshuttled to gluconeogenesis.

Glucagon is a signal for low blood glucose levels. It stimulates breakdown of glycogen, fat, and protein. It inhibits synthesis of glycogen, fat, and protein. Several hours after the intake of dietary carbohydrate, blood glucose levels fallslightly because of the ongoing oxidation of glucose by the brain and other tissues. Although its primary target is the liver, glucagon (like epinephrine) also affects adipose tissue, activating TAG breakdown by activating triacylglycerollipase and liberates free fatty acids, which are exported to the liver and other tissues as fuel, sparing glucose for the brain. The net effect of glucagon is therefore to stimulate glucose synthesis and release by the liver and to mobilizefatty acids from adipose tissue, to be used instead of glucose as fuel for tissuesother than the brain.

During fasting and starvation, metabolism shifts to provide fuel for the brain

The fuel reserves of a healthy adult human are of three types: glycogen stored in the liver and, in relatively small quantities, in muscles; large quantities of triacylglycerols in adipose tissues; and tissue proteins, which can be degraded when necessary to provide fuel. In the first few hours after a meal, the blood glucose level is diminished slightly, and tissues receive glucose released from liver glycogen. There is little or no synthesis of lipids. By 24 hours after a meal, blood glucose has fallen further, insulin secretion has slowed, and glucagon secretion has increased.

Thyroid Hormones

Thyroid hormones (T_4 and T_3) are tyrosine-based hormones produced by the follicular cells of the thyroid gland and are regulated by TSH made by the thyrotropes of the anterior pituitary gland, are primarily responsible for regulation of metabolism. Iodine is necessary for the production of T_3 (triiodothyronine) and T_4 (thyroxine). A deficiency of iodine leads to decreased production of T_3 and T_4, enlarges the thyroid tissue and will cause the disease known as goitre. The thyronines act on nearly every cell in the body. They act to increase the basal metabolic rate, affect protein synthesis, help regulate longbone growth (synergy with growth hormone) and neural maturation, and increasethe body's sensitivity to catecholamines (such as adrenaline). The thyroid hormones are essential to proper development and differentiation of all cells ofthe human body. These hormones also regulate protein, fat, and carbohydrate metabolism, affecting how human cells use energetic compounds. They also stimulate vitamin metabolism. Numerous physiological and pathological stimuli influence thyroid hormone synthesis. Thyroid hormone leads to heat generationin humans. However, the thyronamines function via some unknown mechanismto inhibit neuronal activity; this plays an important role in the hibernation cyclesof mammals and the moulting behaviour of birds.

Iodine is essential for thyroid hormone synthesis

Iodide is actively absorbed from the bloodstream by a process called iodide trapping. In this process, sodium is cotransported with iodide from the basolateral side of the membrane into the cell and then concentrated in the thyroid follicles to about thirty times its concentration in the blood. Via a reaction with the enzyme thyroperoxidase, iodine is bound to tyrosine residues in the thyroglobulin molecules, forming monoiodotyrosine (MIT) and diiodotyrosine (DIT). Linking two moieties of DIT produces thyroxine. Combining one particleof MIT and one particle of DIT produces triiodothyronine. If there is a deficiencyof dietary iodine, the thyroid will not be able to make thyroid hormone. The lackof thyroid hormone will lead to decreased negative feedback on the pituitary, leading to increased production of thyroid-stimulating hormone, which causes the thyroid to enlarge (the resulting medical condition is called endemic colloid goiter).

Circulation and transport

Most of the thyroid hormone circulating in the blood is bound to transport proteins. Only a very small fraction of the circulating hormone is free (unbound) and biologically active, hence measuring concentrations of free thyroid hormones is of great diagnostic value.

When thyroid hormone is bound, it is not active, so the amount of free $T_3/T_4$is what is important. For this reason, measuring total thyroxine in the blood canbe misleading.

Parathyroid Hormone

Parathyroid hormone (PTH), parathormone or parathyrin, is secreted by the chief cells of the parathyroid glands. It acts to increase the concentration of calcium (Ca^{2+}) in the blood, whereas calcitonin (a hormone produced by the parafollicularcells of the thyroid gland) acts to decrease calcium concentration. PTH acts to increase the concentration of calcium in the blood by acting upon the parathyroidhormone 1 receptor (high levels in bone and kidney) and the parathyroid hormone 2 receptor (high levels in the central nervous system, pancreas, testis,and placenta). PTH was one of the first hormones to be shown to use the G- protein, adenylyl cyclase second messenger system. Parathyroid hormone regulates serum calcium through its effects

Region	Effect
Bone	PTH enhances the release of calcium from the large reservoir contained in the bones. Bone resorption is the normal destruction of bone by osteoclasts, which are indirectly stimulated by PTH forming new osteoclasts, which ultimately enhances bone resorption.
Kidney	PTH enhances active reabsorption of calcium and magnesium from distal tubules of kidney. As bone is degraded, both calcium and phosphate are released. It also decreases the reabsorption of phosphate, with a net loss in plasma phosphate concentration. When the calcium:phosphate ratio increases, more calcium is free in the circulation.
Intestine	PTH enhances the absorption of calcium in the intestine by increasing the production of activated vitamin D. Vitamin D activation occurs in the kidney. PTH converts vitamin D to its active form (1,25-dihydroxy vitamin D). This activated form of vitamin D increases the absorption of calcium (as Ca2+ ions) by the intestine via calbindin.

Regulation of PTH secretion

Secretion of parathyroid hormone is controlled chiefly by serum [Ca^{2+}] throughnegative feedback. Calcium-sensing receptors located on parathyroid cells are activated when [Ca^{2+}] is low. Hypomagnesemia inhibits PTH secretion and alsocauses resistance to PTH, leading to a form of hypoparathyroidism that is reversible. Hypermagnesemia also results in inhibition of PTH secretion. Stimulators of PTH includes decreased serum [Ca^{2+}], mild decreases in serum [Mg^{2+}], and an increase in serum phosphate. Inhibitors include increased serum [Ca^{2+}], severe decreases in serum [Mg^{2+}], which also produces symptoms of

hypoparathyroidism (such as hypocalcemia), and calcitriol.

Growth hormone

Growth hormone (GH or HGH), also known as somatotropin or somatropin, isa peptide hormone that stimulates growth, cell reproduction and regeneration inhumans. It is a type of mitogen which is specific only to certain kinds of cells. Growth hormone is a single-chain polypeptide that is synthesized, stored, and secreted by somatotropic cells within the lateral wings of the anterior pituitarygland. The anterior pituitary gland secretes hormones that tend to elevate the blood glucose and therefore antagonize the action of insulin. Growth hormone secretion is stimulated by hypoglycemia; it decreases glucose uptake in muscle.Some of this effect may not be direct, since it stimulates mobilization of free fatty acids from adipose tissue, which themselves inhibit glucose utilization. Growth hormone increases amino acid transport in all cells, and estrogens do this in the uterus. A deficiency of this hormone produces dwarfism, and an excessleads to gigantism.

Regulation of growth hormone secretion

Secretion of growth hormone (GH) in the pituitary is regulated by the neurosecretory nuclei of the hypothalamus. These cells release the peptides Growth hormone-releasing hormone (GHRH or somatocrinin) and Growth hormone-inhibiting hormone (GHIH or somatostatin) into the hypophyseal portal venous blood surrounding the pituitary. GH release in the pituitary is primarily determined by the balance of these two peptides, which in turn is affected by many physiological stimulators (e.g., exercise, nutrition, sleep) andinhibitors (e.g., free fatty acids) of GH secretion. A number of factors are knownto affect GH secretion, such as age, sex, diet, exercise, stress, and otherhormones.

Regulation

Stimulators of growth hormone (GH) secretion include peptide hormones, ghrelin, sex hormones, hypoglycemia, deep sleep, niacin, fasting, and vigorousexercise. Inhibitors of GH secretion include somatostatin, circulating concentrations of GH and IGF-1 (negative feedback on the pituitary and hypothalamus), hyperglycemia, glucocorticoids, and dihydrotestosterone.

Effects of growth hormone

Increased height during childhood is the most widely known effect of GH. In addition to increasing height in children and adolescents, growth hormone has many other effects on the body such as:

- Increases calcium retention, and strengthens and increases the mineralizationof bone
- Increases muscle mass through sarcomere hypertrophy
- Promotes lipolysis

- Increases protein synthesis
- Stimulates the growth of all internal organs excluding the brain
- Plays a role in homeostasis
- Reduces liver uptake of glucose
- Promotes gluconeogenesis in the liver
- Contributes to the maintenance and function of pancreatic islets
- Stimulates the immune system

Clinical significance

Excess

The most common disease of GH excess is a pituitary tumor composed of somatotroph cells of the anterior pituitary. These somatotroph adenomas are benign and grow slowly, gradually producing more and more GH. For years, theprincipal clinical problems are those of GH excess. Eventually, the adenoma may become large enough to cause headaches, impair vision by pressure on the opticnerves, or cause deficiency of other pituitary hormones by displacement.

Prolonged GH excess thickens the bones of the jaw, fingers and toes. Resultingheaviness of the jaw and increased size of digits is referred to as acromegaly. Accompanying problems can include sweating, pressure on nerves (e.g., carpaltunnel syndrome), muscle weakness, excess sex hormone-binding globulin (SHBG), insulin resistance or even a rare form of type 2 diabetes, and reducedsexual function.

GH-secreting tumors are typically recognized in the fifth decade of life. It is extremely rare for such a tumor to occur in childhood, but, when it does, the excessive GH can cause excessive growth, traditionally referred to as pituitarygigantism.

Deficiency

The effects of growth hormone deficiency vary depending on the age at whichthey occur. In children, growth failure and short stature are the major manifestations of GH deficiency, with common causes including genetic conditions and congenital malformations. It can also cause delayed sexual maturity. In adults, deficiency is rare, with the most common cause a pituitary adenoma, and others including a continuation of a childhood problem, other structural lesions or trauma, and very rarely idiopathic GHD. Adults with GHD“tend to have a relative increase in fat mass and a relative decrease in muscle mass and, in many instances, decreased energy and quality of life”.

ENZYMES

The global life depends on a series of chemical reactions. Most of the chemicalreactions proceed too slowly on their own to sustain life. Hence catalysts are required to greatly accelerate the rates of these chemical reactions. In nature enzymes posses the catalytic power to facilitate life processes in essentially alllife-forms from viruses to man. Most of the enzymes retain their catalytic potential even after extraction from the living organism. The above catalytic power of enzyme leads to commercial usage of enzymes.

Characteristics of enzymes

Enzymes are protein catalyst produced by a cell and responsible 'for the high rate' and specificity of one or more intracellular or extracellular biochemical reactions. Enzymes are biological catalysts responsible for supporting almost all of the chemical reactions that maintain animal homeostasis. Enzyme reactionsare always reversible. The substance, upon which an enzyme acts, is called as substrate. Enzymes are involved in conversion of substrate into product. Almost all enzymes are globular proteins consisting either of a single polypeptide or oftwo or more polypeptides held together (in quaternary structure) by non-covalent bonds. Enzymes do nothing but speed up the rates at which the equilibrium positions of reversible reactions are attained. In terms of thermodynamics, enzymes reduce the activation energies of reactions, enabling them to occur much more readily at low temperatures - essential for biological systems. The basic characteristics of enzymes includes

(i) Almost all the enzymes are proteins and they follow the physical andchemical reactions of proteins

(ii) Enzymes are sensitive and labile to heat

(iii) Enzymes are water soluble

(iv) Enzymes could be precipitated by protein precipitating agents such asammonium sulfate and trichloroacetic acid.

Classification

Since earlier days to still date, fanciful names such as pepsin, chymotrypsin, etcwere used to name enzymes. Later the suffix "ase" to the substrate was used toname enzymes. For example the enzymes lactase acts upon the lactate and produces glucose and galactose. The above method is known as "trivial naming"of enzymes. Currently enzymes are grouped into six functional classes by the International Union of Biochemists and Molecular Biology (IUBMB). As per the IUBMB system, each enzyme name starts with EC (enzyme class) followed by4 digits.

The first digit represents the class, the second digit strands for the subclass, thethird digit represents the sub-subclass or subgroup and the fourth digit providesthe particular enzyme.

Sl.No.	Classification	Biochemical Properties
1.	Oxidoreductases	Act on many chemical groupings to add or remove hydrogen atoms. e.g. Lactate dehydrogenase
2.	Transferases	Transfer functional groups between donor and acceptor molecules. Kinases are specialized transferases that regulate metabolism by transferring phosphate from ATP to other molecules. e.g. Aminotransferase.
3.	Hydrolases	Add water across a bond, hydrolyzing it. E.g. Acetyl choline esterase
4.	Lyases	Add water, ammonia or carbon dioxide across double bonds, or remove these elements to produce double bonds. e.g. Aldolase.
5.	Isomerases	Carry out many kinds of isomerization: L to D isomerizations, mutase reactions (shifts of chemical groups) and others. e.g. Triose phosphate isomerase
6.	Ligases	Catalyze reactions in which two chemical groups are joined (or ligated) with the use of energy from ATP. e.g. Acetyl CoA carboxylase

These rules give each enzyme a unique number and specifies a textual name foreach enzyme. Enzymes are also classified on the basis of their composition. Enzymes composed wholly of protein are known as simple enzymes in contrastto complex enzymes, which are composed of protein plus a relatively small organic molecule. Complex enzymes are also known as holo-enzymes. The non- protein component of an enzyme may be as simple as a metal ion or as complexas a small non-protein organic molecule. Enzymes that require a metal in their composition are known as metalloenzymes. Metalloenzymes bind and retain their metal atom(s) under all conditions with very high affinity. Enzymes withlower affinity for metal ion, but still require the metal ion for activity, are knownas metal-activated enzymes. Based on requirement of ATP, enzymes are furtherclassified into two types namely synthetases and synthase. Synthetases are ATP- dependent enzymes catalyzing biosynthetic reactions. Synthetases are enzyme belong to the class 6 (Ligases). Enzymes such as Carbamoyl phosphate synthetase, Arginino succinate synthetase and Glutamine synthetase are examplesof the synthetases group of enzymes. The enzyme class other than ligases includes synthases. Synthases group of enzymes involves in catalyzing biosynthetic reactions that do not require ATP directly. Enzymes such as glycogen synthase and Alanine synthase are examples of synthase group.

Coenzymes

Enzymes may be simple proteins, or complex enzymes. A complex enzyme contains a non-protein part, called as prosthetic group (co-enzymes). Co- enzymes are heat stable low molecular weight organic compound. The combinedform of protein and the co-enzyme are called as holo-enzyme. The heat labile or unstable part of the holo-enzyme is called as apo-enzyme. The apo-enzyme gives necessary three dimensional structures required for the enzymatic chemical reaction. Co-enzymes are very essential for the biological activities of

the enzyme. Co-enzymes combine loosely with apo-enzyme and are released easily by dialysis. Most of the co-enzymes are derivatives of vitamin B complex group of substance. One molecule of the co-enzyme with its enzyme is sufficientto convert a large group of substrate.

Co-enzymes are further divided into two groups. The first groups of co-enzymesare a part of reaction catalyzed by oxidoreductase by donating or accepting hydrogen atoms or electrons. The first group of co-enzymes are also called as co-substrates or secondary substrates. Because they are involved in counter- balance in change occurring in the substrate. The second group of co-enzymesinvolves in reactions transferring groups other than hydrogen.

Role of Coenzymes

The functional role of coenzymes is to act as transporters of chemical groups from one reactant to another. The chemical groups carried can be as simple as the hydride ion ($H^+ + 2e^-$) carried by NAD or the mole of hydrogen carried by FAD; or they can be even more complex than the amine ($-NH_2$) carried by pyridoxal phosphate. Since coenzymes are chemically changed as a consequenceof enzyme action, it is often useful to consider coenzymes to be a special classof substrates, or second substrates, which are common to many differentholoenzymes. In all cases, the coenzymes donate the carried chemical grouping to an acceptor molecule and are thus regenerated to their original form. This regeneration of coenzyme and holoenzyme fulfills the definition of an enzymeas a chemical catalyst, since (unlike the usual substrates, which are used up during the course of a reaction) coenzymes are generally regenerated.

Significance of enzymes

The measurement of enzymes level in serum is applied in diagnostic application.Detection of certain enzymes in the serum indicates that tissue or cellular damage has occurred resulting in the release of intracellular components into theblood. Hence, when a physician indicates that he/she is going to assay for liverenzymes, the purpose is to ascertain the potential for liver cell damage.

Commonly assayed enzymes are the amino transferases: alanine transaminase, ALT (sometimes still referred to as serum glutamate-pyruvate aminotransferase, SGPT) and aspartate aminotransferase, AST (also referred to as serum glutamate- oxaloacetate aminotransferase, SGOT); lactate dehydrogenase, LDH; creatine kinase, CK (also called creatine phosphokinase, CPK); gamma-glutamyl transpeptidase, GGT. Other enzymes are assayed under a variety of different clinical situations but they will not be covered here.

Pancreatic Enzymes

Acute pancreatitis is an inflammatory process where auto digestion of gland wasnoticed with activation of the certain pancreatic enzymes. Enzymes which involves in pancreatic destruction includes α-amylase, lipase etc.,

α-amylase

α-amylase (AMYs) are calcium dependent hydrolyase class of metaloenzymethat catalyzes

the hydrolysis of 1, 4- α-glycosidic linkages in polysaccharides.Molecular weights of AMYs are human plasma ranges from 54 to 62 kDa. Dueto its smaller size they could easily pass the glomeruli of the kidneys and AMYis the only plasma enzyme physiologically found in urine. The normal values of amylase is in range of 28-100 U/L. Marked increase of 5 to 10 times the upperreference limit (URL) in AMYs activity indicates acute pancreatitis and severeglomerular impairment. Pancreatic pseudocyst occurs if the plasma level of amylase activity fails to fall after an attack of acute pancreatitis.

Lipase

Lipase is single chain glycoprotein of molecular weight 48 kDa. Bile salts anda cofactor called colipase are required for full catalytic activity of lipase. Colipase is secreted by pancreas. Lipase is small molecule filtered through theglomerulus and totally reabsorbed by the renal tubules. Lipase is not normally detected in urine samples. The normal value of lipase ranges from 40-200 U/L. Increase in plasma lipase activity indicates acute pancreatitis and carcinomaof the pancreas. So determination of both amylase and lipase together helps in the diagnosis of acute pancreatitis.

Liver Enzymes

The assay of serum enzymes is very useful for the differential diagnosis and monitoring of various liver disorders. Liver enzymes acts as marker of hepatocellular damage, cholestasis and disturbances in the hepatocellular synthesis.

Markers of Hepatocellular Damage

In case of hepatocellular damage, the enzymes which are normally present inside the hepatocytes are released into the blood. Aminotransaminases such as aspartate transaminase (AST) and alanine transaminase (ALT) are routinely used in diagnosis of hepatocellular damages. Transaminases are enzymes involved inthe transfer of an amino group from a 2-amino- to a 2-oxoacid. The 2-oxoglutarate acts as amino group acceptor and the L-glutamate serves as donorin all amino-transfer reactions. The specificity of the individual enzymes derivesfrom the particular amino acid that serves as the other donor of an amino group.

Aspartate transaminase (AST)

Aspartate transaminase is present in high concentrations in cells of cardiac andskeletal muscle, liver, kidney and erythrocytes. Damage to any of these tissuesmay increase plasma AST levels. The normal value of AST for male is <35 U/L and for female it is <31 U/L. Marked increase of AST activity in the rangeof 10 to 100 times the upper adult reference limit indicates myocardial infarctionor acute viral or toxic hepatitis.

Alanine transaminase (ALT)

Alanine transaminase is present at high concentrations in liver and to a lesser extent, in skeletal muscle, kidney and heart. Thus in case of liver damage increase in both AST and ALT were noticed. While in myocardial infarction ASTis increased with little or no increase in ALT. The normal value of ALT is <45U/L and <34 U/L for male and female respectively. In acute viral hepatitis thereis a 100-1000 times increase in both ALT and AST but ALT level is increased more than that of AST.

Markers of cholestasis

Enzymatic markers of cholestasis are membrane bound enzymes. Markers of cholestasis includes alkaline phosphatases, gamma-glutamyltransferase andglutamate dehydrogenase.

Alkaline phosphatases

Alkaline phosphatases are a group of enzymes that hydrolyse organic phosphates at high pH. They are present in osteoblasts of bone, the cells of the hepatobiliarytract, intestinal wall, renal tubules and placenta.

Gamma-glutamyl-transferase (GGT)

Gamma-glutamyl-transferase catalyzes the transfere of the □–glutamyl group from peptides. The activity of GGT is higher in men than in women. In male the normal value of GGT activity is <55 U/L and for female it is <38 U/L. Risein plasma GGT activity is due to infectious hepatitis and induction of enzyme synthesis, without cell damage, by drugs or alcohol.

Glutamate dehydrogenase (GLD)

Glutamate dehydrogenase is a mitochondrial enzyme found in liver, heart muscle and kidneys. Small amounts of GLD are even observed in brain, skeletalmuscle tissue and leukocytes. GLD is increased in serum of patients with hepatocellular damage offering differential diagnostic potential in the investigationof liver disease. GLD is released from necrotic cells and is of value in estimationof the severity of liver cell damage. The GLD upper reference limits are 6U/L (women) and 8U/L (men).

Muscle Enzymes

Clinically important muscle enzymes include creatine kinase and lactate dehydrogenase.

Creatine Kinase

Creatine kinase (CK) is most abundant in cells of brain, cardiac and skeletal.In addition to their abundance in above tissues, it also occurs in other tissues such as smooth muscle. In normal physiological condition the CK activity is 46- 171 U/L (for male) and 34-145 U/L (for female). Serum CK level elevates in all types of muscular dystrophy. Quite high values of CK are noted in viral myositis, polymyositis and similar muscle disease. Under the circumstances of neurogenic muscle disease such as: myasthenia gravis, multiple sclerosis and Parkinsonism, the level of serum CK is normal. CK consist of two protein subunits, M (for muscle) and B (for brain) and exist as three different isoformsnamely BB (CK-1), MB (CK-2) and MM (CK-3). CK-MM is the predominantisoenzyme in skeletal and cardiac muscle and is detectable in the plasma of normal subjects. CK-BB is present in high concentrations in the brain and in the smooth muscle of the gastrointestinal and genital tracts.

Lactate Dehydrogenase

Lactate dehydrogenase (LD) catalyses the reversible interconversion of lactateand pyruvate. LD has a molecular weight of 134 kDa and is widely distributedin the body, with high concentrations in cells of cardiac and skeletal muscle, liver, kidney, brain and erythrocytes. Five isoforms (LD-1 to LD-5) of LD are existing. The normal physiological limit of LD is 180-360 U/L.

Other clinically important enzymes includes acid phosphatase, glucose -6- phosphate dehydrogenase, cystathionine α-synthase and sphingomyelinase.